

Mobilizing Science to Break Yield Barriers

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ABSTRACT

Yield barriers must be broken. The diminished stock of staple foods, higher grain prices, and increases in production failing to keep up with demand, coupled with 80 million people being added to the world population every year, suggests that we are on a collision course with famine unless greater investments are made in research and development, as well as education. Genetic improvement of staples has accounted for more than half of the past increases in yields. Fortunately, a revolution in genetic knowledge is co-evolving with the increased demand for food, feed, fiber, and fuel. Utilizing genetic diversity has been a mainstay of past production improvements. High throughput DNA sequencing, the related bioinformatics, and a cascade of genetic technologies can now be employed to detect previously hidden genetic variability, to understand gene functions, to make greater use of accessions in germplasm banks, and to make breeding schemes more efficacious. The involvement of outstanding scientists who can bring interdisciplinary ideas to the question of how to break yield barriers must be part of the strategy. Educational programs at all levels, even high school, should emphasize the opportunities in international agriculture to build a cadre of dedicated scientists for the future.

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Abbreviations: MAS, marker-assisted selection; QTL, quantitative trait locus.

GENETIC variability hidden in our plant and animal genomes represents an international treasure important to everyone living on this planet. Natural selection of this variability is the basis for the evolution of all living things, as proposed by Darwin in *Origin of the Species* 150 yr ago (Darwin, 1859). Selection of phenotypic traits at the hands of humankind for the past 10,000 yr now allows the production of enough food for 6.5 billion people. No one knows if this hugely successful past can foretell the future. Fortunately, undiscovered variability lurks in every genome to an extent that we could not even contemplate before the advent of genomics, the main theme of this article. Genetic variability is worthless in agriculture if not utilized; once discovered the variability needs to be understood and incorporated into breeding programs. Rapid means to detect the variability, identify the gene function(s), and progress through a breeding program is key to breaking yield barriers. With the past and modern advances in genetics and the advent of transgenic technology, the circle is complete in making available the wonderful arrays of variability manifested in the natural world.

Yield barriers can be viewed from at least two perspectives. A yield barrier may exist because increases in production are not keeping pace with the increases in demand. Another viewpoint is that a yield barrier is present when physiological maxima have

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been reached. Most technologies reported herein apply directly to the first perspective and indirectly to the second. Transferring C4 photosynthesis, for example, would apply directly to the second viewpoint.

The importance of breaking yield barriers is paralleled by the rate of the world population increase. The world population is expanding at a rate of about 1 billion people every 12 to 14 yr, or about 80 million people per year. T.J. Higgins, the Commonwealth Scientific and Industrial Research Organization's (CSIRO) deputy chief of plant industries, recently said that "population and rising wealth could mean an extra 10 billion tons of food consumed each year by 2025" (McKenzie, 2009). Furthermore, the effect of population increase is much more pronounced in the developing world where applications of some of the new technologies are just commencing to be applied to food production.

The objective of this meeting as stated in the Science Forum 2009 announcement was as follows: "The Forum intends to stimulate provocative and challenging discussion, with a forward-looking perspective on research and partnership needs to increase the resilience and the productivity of agricultural and natural resource systems (CGIAR, 2009)."

This report principally focuses on technology and the progress expected from these technologies. Bruce Alberts, the former President of the National Academy of Sciences and current editor-in-chief of *Science* magazine, recently stated in an editorial on breakthroughs of the year: "The scientists who achieved each of this year's breakthroughs exploited techniques and instrumentation that were unimaginable when I began my life as a scientist in the 1960s. To mention only a few: computational speeds and methods, detectors, telescopes, DNA sequencers, and recombinant DNA technologies. These new technologies are created from the knowledge of the natural world generated by previous scientific and technical advances. Therefore, the more we know, the more we can discover, and the pace of scientific discovery constantly accelerates (Alberts, 2008)." It seems clear that we must embrace traditional approaches but complement them with new technologies to maximize the prospect of breaking the yield barrier in food staples.

High food prices have left millions of people hungry, with this group of people now accounting for 14% of the world's population. Oxfam reported "current severe food shortages in Afghanistan, Ethiopia, Kenya, Mozambique, and Zimbabwe are evidence that the global food crisis is far from over. Even before recent price rises, there were over 850 million people classified as undernourished. Now, there are nearly a billion, as a result of the price rises, alongside other factors such as political instability and conflict (Oxfam International, 2009)."

Nearly two-thirds of the world's undernourished people live in Asia and one-third in Sub-Saharan Africa. Speaking on behalf of the developing countries, the Bangladesh

minister of Agriculture, C.S. Karim, indicated that "we need to find more powerful gene revolution tools for food, feed, medicine, renewable energy, and other human needs" (ISAAA, 2008). It is encouraging that one of the world's largest agricultural companies, Monsanto, has reconfirmed that they believe their goal of doubling crop yields by 2030 is attainable (Wanzek, 2008). Although much more than technology is needed to increase food production, including credit, availability of inputs such as nutrients and water, markets, and other externalities, about half of the increases in productivity in major crops over the past several years have been due to the genetic improvement of these crops. Thus, this article will focus on genetic technologies believed to be important for the future of agriculture. Employment of new agronomic practices is also essential and may need to be coupled with the breeding procedure for maximum impact.

History shows that as the population increased, agricultural practices and technology allowed more food to be produced on less land. The assumption is made that breaking the yield barrier will again mean that more food be produced on less land (Table 1).

Maize provides a good case study to show how plant breeding technology has allowed a 1% or better gain per year. Figure 1 (used with permission of A. Forrest Troyer; E. Wellin and F. Troyer, unpublished data, 2008) shows the steady improvement in corn yields since new technologies were introduced. Note that the slope increases with the advent of each new technology— from open-pollinated populations, to double crosses, to single crosses, to biotech hybrids.

Productivity increases for the important staples rice and wheat, however, are not nearly as impressive as for corn. The average increase in rice production in the 1980s was 3.1% per year but in the 1990s it decreased to 1.4% per year. Even worse, the 2000s have seen increases of only 0.8% per year. Likewise, wheat production increased at a rate of 2.9% per year in the 1980s but only 0.9% in the 1990s and 0.4% in the 2000s (Ziska, USDA-ARS, Beltsville, MD, personal communication, 2009). Why is this the case? Pardey et al. (2006) argues that reduced public investment in agricultural research may be a principal factor. The private sector investment in maize may account for its success.

As stated by Cliff Weil (Johal et al., 2008): "The success of a breeding program depends on having adequate diversity in the germplasm. However, as advanced breeding stocks and materials are generated, one casualty is the diversity itself. As a result, breeding programs in many crop species have reached a point of diminishing returns and it is feared that unless new diversity is infused into the breeding germplasm, we face catastrophic reductions in crop productivity if the climate turns adverse. Although some scientists favor transgenic approaches, a 'back to nature' approach to genetic diversity may prove faster and more effective. Wild and exotic relatives of crop plants

hold a wealth of alleles that, if we can find them, can help break yield barriers and enhance tolerance to stresses.”

A 2009 National Research Council Report entitled “Emerging technologies to benefit farmers in Sub-Saharan Africa and South Asia” reports on 60 emerging technologies and lists 18 as priorities for immediate development (National Research Council, 2009). Technologies considered the most important are those that “(i) manage the natural resource base supporting agriculture; (ii) improve the genetic characteristics of crops and animals; (iii) reduce biotic constraints (such as disease, pests, and weeds); and (iv) provide affordable, renewable energy for farmers.” This article supports those opinions, but due to space limitations obviously cannot directly touch on all of them. The analysis presented here was deliberately prepared before reading the NRC report to present an independent viewpoint on breaking the yield barrier. The two reports are both supportive and complementary of each other. This article perhaps provides more emphasis on breeding schemes, variability in parental materials, and investing in human capital.

VARIABILITY

Detection of variability depends in large part on progress in DNA sequencing. The initial deciphering of

Table 1. Historical information on the amount of land required to provide food for the size of the population at different times (cf. CropLife Intl. 2007–2008 Annual Report).

Date	Population	Land/Person
1960	3.0 billion	4.3 ha (10.8 ac)
1980	4.4 billion	3.0 ha (7.5 ac)
2000	6.0 billion	2.2 ha (5.5 ac)
2020	7.5 billion	1.8 ha (4.5 ac)
2050	9.2 billion	???

the human genome cost \$3 billion and took 13 yr. Two years ago, James Watson’s genome was sequenced in 2 mo for \$2 million. Several plant species now have been sequenced such as *Arabidopsis*, rice, poplar, grapes, sorghum, maize, soybean, etc. (see <http://www.jgi.doe.gov> [verified 14 Dec. 2009]). The sequencing of *Arabidopsis* required \$70 million and about 7 yr. Maize, which has about 2300 Mb of DNA as opposed to the much smaller *Arabidopsis* 140 Mb genome, will cost half as much as *Arabidopsis*. New sequencing procedures now can give sequencing reads of 400 to 500 bases or longer and generate more than 1 million sequencing reads per 10-h instrument run (see <http://www.454.com> [verified 14 Dec. 2009]) Newer technologies such as the Single Molecule Real Time (SMRT) DNA sequencing technology (see

Fig. 1. Average U.S. corn yields and kinds of corn, Civil War to 2007; periods dominated by open-pollinated varieties, four-parent hybrids, two-parent hybrids, and genetically modified hybrids are shown. *b* values (regressions) are yield gain per year (kg/bu). USDA data compiled by E. Wellin and F. Troyer.

www.pacificbiosciences.com [verified 14 Dec. 2009]) may allow the sequencing of *Arabidopsis* for \$70. About 15 Gb of DNA may be sequenced in a 7-d run in the future at a cost of about \$0.50 per million bases.

If understanding and utilizing diversity is one of the keys to breaking the yield barrier, why don't we think big and sequence the entire rice germplasm collection of 100,000 accessions in the International Rice Research Institute (IRRI) genebank? At \$0.50 cost per million bases and rice with 430 Mb of DNA, the cost would be \$200 to \$300 per accession, or a total of \$20 to \$30 million. At first, this bold proposal may appear unrealistic. However, consider the fact that there is already a 1000 Genomes Project for humans and a proposed 1001 Genomes Project for *Arabidopsis*. The goal of the 1001 Genomes Project is to discover whole-genome sequence variation in *Arabidopsis*. Although such information in *Arabidopsis* will provide important parameters for understanding variability in other species, much of the information is species- and strain-specific.

As scientists, we must remember that technology advances—especially in genetics—faster than most of us would ever contemplate. Even with the advances in genomics and other “omics,” some still complain that the new technology has not solved many problems. This will no doubt be a fading viewpoint as agricultural applications become apparent, especially in rice. With regard to DNA sequencing, the sequencing of the 5386-base bacteriophage PhiX174 in 1977 was a highlight in science. Considering the technology used to sequence PhiX174, completion of the *E. coli* chromosome would have required a thousand years and the human genome would have taken a million years (Stein, 2008). Resequencing is a rapidly growing activity and will be extremely enhanced as the next generation sequencing comes along (Liszewski, 2009). In resequencing, the sequence of many lines are compared to the sequence of the reference genome to find differences that provide a multitude of useful new molecular genetic markers. Pioneer Hi-Bred International has resequenced more than 600 key lines for 10,000 sequences (Mark Cooper, Pioneer Hi-Bred Intl. personal communication, 2009).

Sorting out useful information is a challenge when dealing with such large amounts of data. However, bioinformatics approaches are becoming more and more user friendly. A new web-based program called TARGeT (formerly called TATE) will automatically identify and list gene family members from a genomic database and show a phylogenetic tree. For example, TARGeT has been used to find the homologs of the ascorbate peroxidase gene family in maize, rice, and sorghum. TARGeT can find the known homologs in this gene family in the 430 Mb genome of rice and draw a phylogenetic tree—and it takes only 75 s! (Han et al., 2009). Twenty-eight homologs were found across all three genomes (totaling thousands of megabases)—and that only took about 10 min.

For maize, if the genetic map location of a gene is known, the Maize GDB Genome Browser will show the sequence in that region using the Locus Lookup tool (Sen et al., 2009). For the waxy (*wx1*) locus, for example, the tool shows that this mutation is between 28,694,400 and 28,699,300 on chromosome 9. The gene thus is targeted to a 4900 base pair region of the genome.

Many genomics-related software programs are available for testing a wide variety of questions (National Research Council, 2008; see <http://www.nap.edu> [verified 14 Dec. 2009]). A program called MAGIC (Mutant-assisted gene identification and characterization) searches for genes that either enhance or diminish certain traits (Weil, 2008). This approach utilizes the introgression of a mutant into various backgrounds and identifies QTLs (quantitative trait loci) that modify the expression of that mutant trait.

Beyond identifying genes and their known functions, databases exist that assist in understanding various plant metabolic pathways. The PMN (Plant Metabolic Network) database (Available at <http://www.plantcyc.org/> [verified 14 Dec. 2009]) helps in understanding the chemical reactions that make up metabolic pathways, such as the conversion of carbon dioxide, transportation of nutrients, and responses to the environment (Weil, 2008).

Natural sequence variability in crop and animal genomes is vast. The resequencing approach identifies single nucleotide polymorphisms (SNPs) between and among different strains. It is not uncommon now to find a million differences that can be used as molecular genetic markers and identified by simple PCR (polymerase chain reaction) procedures. SNPs can be used in association mapping or other mapping approaches. Thus, the variation that can be used to locate the portion of a genome associated with the expression of a specific trait (Buckler et al., 2008) is readily available once the resequencing is accomplished.

Diversity is a key feature to plant improvement. The extensive germplasm collections maintained around the world are important in protecting future food supplies. They need to be maintained in viable condition, evaluated for traits of interest, and rejuvenated at appropriate times. But even with the proper care of these materials, they are generally difficult to use due to the fact that a cross brings in all of the deleterious factors along with the genetic factor of interest. Crosses between adapted varieties and exotic accessions require a long time to derive useful genetic material; this has deterred plant breeders from using exotic germplasm (Bernardo, 2009). Procedures need to be developed that allow the transfer of much less than the whole genome from the germplasm accession. Transgenic technology as practiced today only inserts one or a few genes at a time. Expansions of the technology are needed to allow the transfer of at least the “selective sweep” of genes associated with a particular region. Of course, the extensive availability of SNPs allows the

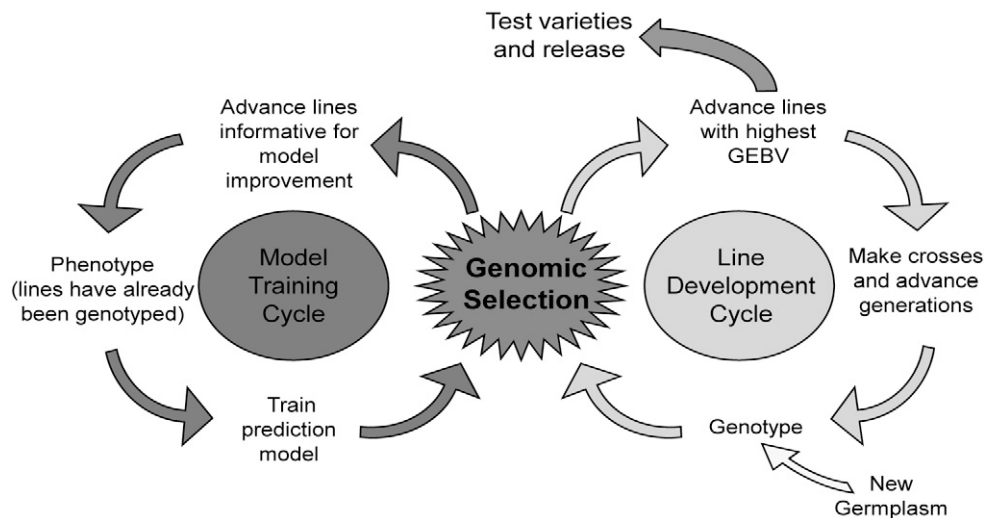


Fig. 2. Breeding approach using genomic selection which is shorter than a conventional program largely by eliminating the phenotypic evaluation of parents for the next cycle (GEBV, genomic estimated breeding value). Used with permission from Jean-Luc Jannink (Cornell University; J.-L. Jannink, unpublished data, 2008).

monitoring of segments of the genome to facilitate the introgression of chromosome blocks. The identification of introgressed markers followed by the subsequent comparison with the whole-genome sequence will identify the chromosomal regions that are now transferred from one strain to another (Stupar, Univ. Minnesota, personal communication, 2009).

Breaking the yield barrier can be achieved in two ways: Increasing “attainable yield” such as from insect resistance, etc., and from “potential yield” such as raising the base yield due to changes in physiological processes. Transgenics thus far appear to have raised operational yield. Interestingly, improvements in the heterotic mechanism(s) with traditional breeding have not been documented; much of the yield improvements have been due to increased inbred yield (Duvick, 1999). Plant population density, leaf uprightiness, lodging resistance, and “stay green” clearly have been important. To date, manipulation to increase photosynthesis does not appear to be involved in raising yields.

GENE FUNCTION

Systems for tagging genes are now quite robust. About 250,000 T-DNA insertions are available in *Arabidopsis* (Phillips et al., 2004). This reflects an insertion about every 500 bp in the genome. According to a report by Eric Vollbrecht (Vollbrecht et al., 2008), about 2000 maize lines now exist that have a uniquely located endogenous *Ds* (McClintock’s Disassociation element) insertion, such that 85% of the genetic map space is within 4 cM or less of a placed *Ds*. Given the propensity of *Ds* for local transposition, targeted disruption of most corn genes is now possible (see <http://www.plantgdb.org/prj/AcDsTagging/> [verified 14 Dec. 2009]).

Testing for expression of genes across the genome in one experiment has become possible for many species. A tremendous number of sequences can be assayed for expression. The various DNA chips manufactured by Affymetrix (<http://www.affymetrix.com> [verified 14 Dec. 2009]), for example, for *Arabidopsis*, barley, citrus, cotton, maize, and Medicago allow thousands of genes to be assayed for expression. Nimblegen (<http://www.nimblegen.com> [verified 14 Dec. 2009]) has an array for maize with 2 million probes. Of course, expression depends on the environment, so the conditions of plant growth are important and, ultimately, should reflect the targeted field situation.

BREEDING

Precision phenotyping also will be a key to breaking the yield barrier. High throughput genotyping is of little value if the phenotype is not accurately measured. The measurement of yield requires sophisticated experimental designs involving large populations, replication across time and space, and appropriately adjusting for moisture and other factors. Whether the trait is a visible phenotype or biochemical in nature, the accuracy of the data largely determines the validity of the interpretations. High throughput phenotyping even in the field will be increasingly common (Montes et al., 2007). What might be called “phenotype science” needs to be expanded in theory and practice and emphasized in all programs across the world.

Resurgence of doubled haploid breeding has occurred in perhaps 80% of the corn companies. With the new high throughput genotyping platforms and the extensive phenotyping efforts, the application of these technologies is even more cost effective with doubled haploids since the material is true breeding. Other factors making the doubled haploid breeding method attractive include the development of an inducer line called RWS that generates

an average of over 8% haploids on an ear, more efficient chromosome doubling methods utilizing nitrous oxide, new genetic markers, etc. (Touraev et al., 2008), as well as the efficiency of introgressing transgenes—especially stacked transgenes (Rober et al., 2005). Training in the doubled-haploid method of breeding may be important in breaking yield barriers. Different systems of producing haploids exist with different crops; haploids can be produced at high efficiency at some point in the life cycle of different species. Of course, haploids also can be valuable in polyploid plants, such as the tetraploid potato where a haploid is essentially a diploid with much simpler genetics than the tetraploid parent (Mendiburu and Peloquin, 1977).

Genomic selection, where high-density marker information—if not whole genome sequences—allow the prediction of breeding values, is being considered by several plant breeders. All marker information is incorporated into the prediction model (Heffner et al., 2009; Bernardo, 2009). Although only based on simulations at this point, the correlation of the estimated breeding value and the true breeding value may be as high as 0.85. This approach may decrease the need for extensive phenotyping in certain portions of the breeding process. Genomic selection appears to be effective even for low heritability traits controlled by many genes. The marker-assisted selection (MAS) strategies currently employed seem to work best for genes with major effects and, thus, are not very efficient for most traits of interest which are generally polygenic. Schaeffer (2006) estimated that the rate of gain could be increased two-fold and reduce the cost (perhaps by 92%) of progeny tests. Several recent papers on sequencing and mapping indicate that such strategies will be increasingly available for agricultural animal species (The Bovine Genome Sequencing and Analysis Consortium et al., 2009; The Bovine hapMap Consortium, 2009; Chessa et al., 2009).

The purpose of phenotyping in this breeding approach is to estimate, or re-estimate the effects of the various markers or sequences (Fig. 2).

Biotech varieties created through transgenic technologies theoretically allow the capturing of any genetic variability of interest no matter its source. It seems prudent in regard to breaking the yield barrier to advocate both modern conventional breeding schemes and transgenic approaches even though genetic engineering is not totally acceptable around the world. Many traits can be approached by both technologies, such as the extremely important trait of drought tolerance. For example, the WEMA (Water Efficient Maize for Africa) program hopes to have their first tolerant varieties available in 6 to 7 yr, with drought tolerant transgenic varieties to be released commercially in the U.S. in 2012 and in Sub-Saharan Africa by 2017 (James, 2008). This work is supported by the Bill and Melinda Gates Foundation (\$42 million)

and the Howard G. Buffett Foundation (\$5 million) and reflects a highly collaborative project led by the African Agricultural Technology Foundation and involving CIMMYT, Monsanto, farmer groups and seed companies in Kenya, Mozambique, South Africa, Tanzania, and Uganda. Many of the private sector companies are also working toward similar goals (Edmeades, 2008). Monsanto recently submitted a request to USDA for release of the first corn tolerant to drought by transgenic technology. Their field trials indicate a 6 to 10% yield advantage across a range of genetic backgrounds under a stress that reduces yields by 50% (AgBioView, 2009).

Another example where a dual approach is prudent is in regard to submergence-tolerant rice. Flooding causes the loss of 4 million tons of rice each year—enough to feed 30 million people. Normal rice cannot tolerate complete flooding more than about 3 d, whereas rice with the *sub1* gene from indica rice can survive 2 wk or more. This gene was identified about 13 yr ago and has now been incorporated by MAS into several megavarieties by IRRI and into local varieties by national programs. IRRI is able to transfer the gene into other varieties in 2.5 yr with the assistance of MAS. Trials by IRRI in Bangladesh, Vietnam, Cambodia, and India have given positive results—saving the crop and providing income. This gene has been cloned (Xu et al., 2006) and could be transferred subsequently by transgenic technology.

Golden rice is an example where genetic variability for a trait of interest was not available in the target species, namely rice. Even though the rice germplasm bank is very comprehensive with over 100,000 entries, rice with higher levels of β -carotene did not exist. However, Potrykus and Beyer (Ye et al., 2000) were able to utilize genes in the daffodil and the *Erwinia* bacterium to complete the carotenoid pathway in rice. Golden rice 2 has the yellow endosperm gene from maize incorporated and gives a much higher carotenoid level. IRRI anticipates releasing Golden rice 2 in 2012 (Aguiba, 2009). The Rockefeller Foundation will provide funding to help guide golden rice through national regulatory approval processes in Bangladesh, India, Indonesia, and the Philippines (Rodin, 2008).

Post-harvest preservation of the agricultural product can alleviate the need to break the yield barrier. The African Journal of Food, Agriculture, Nutrition, and Development (Oniang'o, 2009) reported a story in the local press that \$8 million worth of maize was destroyed by court order due to high levels of aflatoxin. Given that about 10 million people in Kenya were chronically short of food (out of 36 million), such loss of food can be devastating— and can be prevented.

Genetics can play a role in reducing postharvest losses. Interesting examples relate to aflatoxin. For example, in groundnut, stilbene phytoalexin is produced in response

to fungal infection (Sobolev et al., 2006). The aflatoxin gene cluster of *Aspergillus* has been identified and studied extensively (Carbone et al., 2007). The lipoxygenase enzymes (LOXs) also are believed to play a role in *Aspergillus* infection (Tsiatsigiannis et al., 2005).

In general, crop management and storage structures and practices are important in avoiding dangerous aflatoxin levels.

Frontier projects with high risk but high reward—and usually requiring partnerships as do most international agriculture projects—need to be part of the portfolio of approaches to break the yield barrier. Crop species differ in the efficiency of the photosynthetic process. In C3 plants, the direct carboxylation of ribulose-1,5-bisphosphate is achieved via the enzyme RUBISCO. This enzyme causes the loss of fixed CO₂ via photorespiration, which can decrease the photosynthetic potential by 40% (Matsuoka et al., 2001). C4 plants concentrate CO₂ in the bundle sheath cells—the locus of RUBISCO—therefore preventing the loss via photorespiration of previously fixed carbon.

Nineteen families of flowering plants have C4 photosynthesis; the evidence indicates that C4 photosynthesis has evolved naturally over 50 times (Sage and Sage, 2007). This and the finding of species with intermediate forms of photosynthesis inspired the creation of a global consortium to work toward the development of a C4 rice that is expected to be much more efficient. The Bill & Melinda Gates Foundation has provided a grant of \$11 million in support of the consortium led by IRRI; the goal is to develop rice plants that can produce 50% more yield using fewer inputs such as nitrogen fertilizer and water with much greater efficiency. This is a high risk long-term project requiring at least 10 yr (Sheehy et al., 2007).

An interesting approach is via the crossing of C3 and C4 species. The generation of chromosome addition lines by crossing oats by corn followed by embryo rescue (Phillips and Rines, 2009) represents one approach for transferring C4 characteristics to a C3 species. A complete set of oat–corn addition lines have been produced where each corn chromosome (1 through 10) is present individually in an oat background. Over 650 radiation hybrid lines have been produced from these addition lines via irradiation providing lines that have only a segment of a corn chromosome in the oat background. Studies have shown that genes for key enzymes in photosynthesis are present in different addition lines as expected from earlier mapping data. These genes from corn express both the RNA and the protein in the oat background. CO₂ compensation point analysis of the individual addition lines for corn chromosomes 6 and 9 was normal; however, the double addition of 6 plus 9 had a significantly lower compensation point but it was still more like oat than corn (Kowles et al., 2008).

Duplications arising from ancient tetraploidy can be observed in crop species such as corn and rice even though

their behavior is as diploids. One hundred fifty years ago in *Origin of the Species*, Charles Darwin said “It is not the strongest species that survive or the most intelligent, but the ones who are most responsive to change.” Could it be that duplicate genes and chromosome regions or whole genomes provide that ability to be responsive to change? The original function of the gene can be maintained while the duplicate copy is free to change and provide a new or related function. Or the expansion of a repeated genic region can lead to phenotypic changes. Triplet repeat expansions underlie many human genetic disorders and phenotypic variation in microbes as well as plants (Sureshkumar et al., 2009). Or do highly homologous duplicate genes produce products that interact to increase productivity? Since duplicate genes exist in all of our agricultural species, do these interact to provide hybrid vigor in outcrossing species or simply increases in productivity in selfing species?

De novo variation allows variation to appear in progenies that is not present in the parents. Today, we are aware of several mechanisms by which this can occur; these include point mutations, intragenic recombination, transposable elements, epigenetic variation, gene amplification, and others (Rasmusson and Phillips, 1997).

Understanding epigenetic variation may lead to a better understanding of intrinsic yield. The complexity of phenotypes in crop plants and the interactions with the environment (G x E interactions) probably cannot be explained by structural genetics alone but must be considered together with DNA alterations occurring through potentially reversible changes such as histone modifications, methylation, and imprinting. A genomic methylation dataset will be possible reflecting various tissues and developmental times. A NIH Epigenome Roadmap project has been funded— involving four U.S. genome centers— to map epigenetic sites in about 100 cell types (Stein, 2009).

How the genetic background is manifested in terms of variability in a particular trait is not understood, yet the phenomenon is common and can reflect major differences in expression of the phenotype. For example, early flowering in maize is considered a highly heritable trait but shows extensive genetic background effects. The *vgt1* gene of maize had a 10-d effect on maturity in the material utilized for QTL analysis and gene cloning (Phillips et al., 1993). The effect can be absent in certain backgrounds and much greater than 10 d in others. The responsible genic segment is a noncoding sequence acting on a flowering gene 70 kb distant (Salvi et al., 2007).

How noncoding sequences are involved in G x E interactions is yet to be learned; however, any information on the molecular basis of G x E interactions will be important in breaking yield barriers.

Double-stranded break-enhanced gene targeting allows specific genes to be modified in specific ways. Endonucleases can be designed to produce double-stranded breaks at

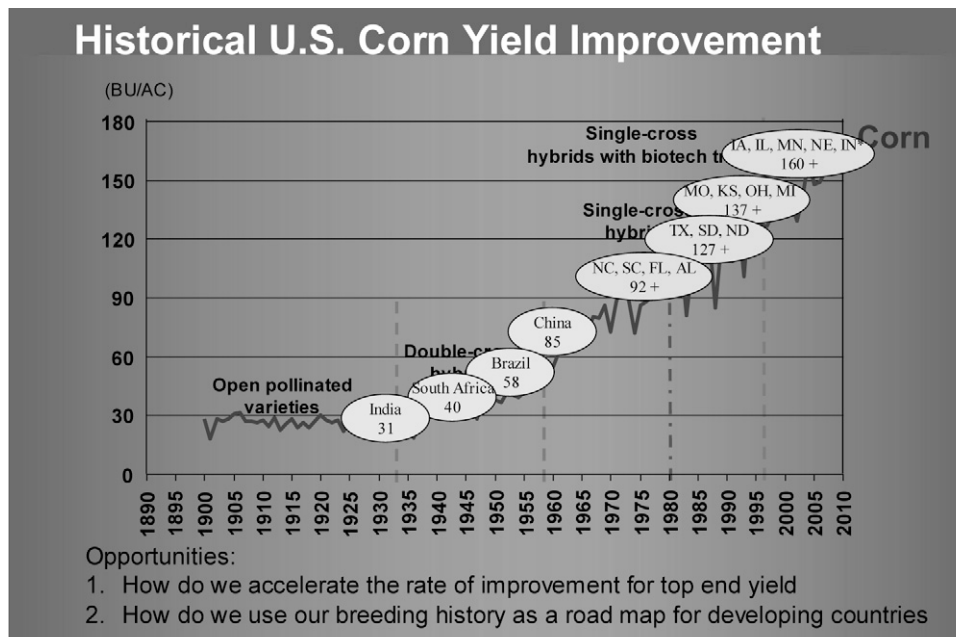


Fig. 3. U.S. corn yield improvement over the past century and more. Used with permission from Geoff Graham (Pioneer HiBred Intl.; G. Graham, unpublished data, 2009).

predetermined sites. Such enzymes can cause a single cleavage within a genome as large as the human genome— which is the same size as that of corn. Also, the genome of a pathogen can be the target without affecting the host genome. Precision Biosciences (<http://www.precisionbiosciences.com> [verified 14 Dec. 2009]) has the “directed nuclease editor” that enables the production of such “homing endonucleases.” Zinc-finger nucleases are increasingly being proposed as a more reliable way of modifying genes, such as for the generation of herbicide-resistant plants (Townsend et al., 2009). A Zinc-Finger Consortium is making the technology openly available to researchers at no cost.

Learning from the past should help us enhance yields in the future. Considering the response of corn yield versus time, one can consider where different countries are on that graph. India currently produces 2000 kg/ha (31 bu/ac), which is the same point the U.S. corn yields were at in about 1930. South Africa reflects about 1940 technology at 2500 kg/ha (40 bu/ac), Brazil is at 1950 yields, and China is at 1960 U.S. production levels. Even different states in the U.S. differ in their average yields (Fig. 3). The question is: Can we apply what has been learned in the improvement of corn yields in the U.S. to developing countries and greatly speed up the breaking of their current yield barrier? Does the answer lie in science, education, input availability, credit, workforce, politics, social structure, or other factors that impact food security?

FUTURE

Investing in human resources to capture new ideas and enthusiastic dedication is perhaps one of the

most important approaches to breaking the yield barrier. Upon learning of the needs in developing countries, today’s students recognize the extensive opportunities that await them to make a difference. Because many students cannot spend extended time away from their current studies, a program was developed to provide short-term exposure to international agriculture via IRRI (Phillips et al., 2008). A course on rice biology called ‘Rice Research to Production’ also is offered, funded by the National Science Foundation (see <http://beta.irri.org/training/home.php> [verified 14 Dec. 2009]). Another recent opportunity is the Monsanto Beachell-Borlaug International Scholars Program offering opportunities for Ph.D.-level training in rice and wheat breeding, connecting developed and developing countries (see http://www.monsanto.com/responsibility/sustainable-ag/produce_more/beachell_borlaug/goals.asp [verified 14 Dec. 2009]). Programs such as these are needed to bring fresh ideas to the yield barrier issue. Even starting younger at the high school level can be quite effective. The World Food Prize Global Youth Institute identifies high school students who then spend a summer at an international agricultural research institute (see <http://www.worldfoodprize.org> [verified 14 Dec. 2009]). There is little question that the experience turns their lives around causing them to have an intense interest in food production and poverty in developing nations.

The extensive interactions of the CGIAR centers with advanced research institutes and national programs (NARES) are highly collaborative and have paid huge dividends. These partnerships together with appropriate funding mechanisms such as the Challenge Grants (see

<http://www.cgiar.org/impact/challenge/index.html> [verified 14 Dec. 2009]) need to be fostered, based on many years of positive experiences.

A combined effort of the Rockefeller Foundation and the Bill and Melinda Gates Foundation has established the Alliance for a Green Revolution in Africa (AGRA). Training will be a major component, with the program expecting to train approximately 120 Ph.D.-level plant breeders over the next decade. For example, AGRA recently announced a new partnership with the University of Ghana, Legon, and the strengthening of a program piloted at the University of KwaZulu-Natal in South Africa to create a critical mass of breeders to alleviate Africa's food deficit (see http://www.rockfound.org/about_us/news/2007/0919agra_pr.shtml [verified 14 Dec. 2009]).

To break yield barriers, truly interdisciplinary approaches need to be implemented, with many to be found outside of the traditional agricultural institutions. How do we make outstanding scientists in complementary fields aware of the opportunities? Publishing "invitational articles" in *Science* and other journals is one approach indicating the need and the rewards of such research (Phillips, 1997). Of course, there needs to be the prospect of longer-term funding.

In an April 2008 editorial in *Science*, Nina Federoff, senior scientific advisor to the U.S. Secretary of State, wrote: "A new Green Revolution demands a global commitment to creating a modern agricultural infrastructure everywhere, adequate investment in training and modern laboratory facilities, and progress toward simplified regulatory approaches that are responsive to accumulating evidence of safety. Do we have the will and the wisdom to make it happen?" (Federoff, 2008).

Bill Gates suggested in a speech to the 2008 Davos World Economic Forum that we need a new business model. The model should include the motivation to help humanity and development driven by the profit motive. He called this "Creative capitalism"—coupling idealism and an altruistic desire to help others (Federoff, 2009).

Former U.S. Vice President Al Gore said at the 2009 American Association for the Advancement of Science meetings that: "There is nothing more powerful than an idea whose time has come". He also said that: "If you want to go quietly, go alone. If you want to go far, go together."

Pandit Jawaherlal Nehru said in a famous statement that "Everything else can wait, but not agriculture." Let us not wait any longer but promptly move ahead in helping those in need. The theme in this article is that agricultural applications follow the biology. We are generating, for example, genome instruction books in regard to plants, animals, and microbes—how will we use them in agriculture around the world? Remember, to answer a question, the question must first be asked.

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