

DEVELOPING CORN HYBRIDS WITH NEW TRAITS

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Corn is the primary starch crop grown in the U.S. and is used primarily as an animal feed (57 percent of the crop, see Figure 1). Export (19 percent) and ethanol production (11 percent) represent the next two largest uses of the corn crop. Corn as a commodity crop has been bred primarily to have high grain yield (starch) and good agronomics. As corn use becomes more specialized — for example, corn used for ethanol production — there has been interest in adding new traits to corn to make it more suitable for the special use, and perhaps to obtain a price premium. There are many examples and types of specialized traits, and the purpose of this paper is to provide a brief overview of how breeders introduce new traits into corn hybrids. Our discussion will include both transgenic and non-transgenic traits.

Reproductive Biology of Corn

To understand how a crop is bred, we must first understand the crop's reproductive mechanism. Corn is a monoecious plant, meaning that the male flowers (tassels) are on a separate part of the plant from the female flowers (ear shoots). The pollen is dispersed from the tassels by wind to the silks of ear shoots. Corn is a nearly 100 percent outcrossing species, meaning that the silks on the ear shoots are primarily pollinated by pollen grains from other plants. A typical corn plant produces approximately 4,500,000 pollen grains (Westgate et al., 2003) and 300 to 600 silks (Westgate et al., 2003).

The flowers (tassel and ear shoots) of corn are large and are easily amenable to making hand pollinations. Breeders make hand pollinations in corn by covering the ear

shoots with small glycine bags before the silks emerge. Once the silks have emerged on a plant that is to be used as a female, a large paper bag is placed on the tassel of the male plant the day before the pollination will be made. The pollination is then made by simply collecting the pollen in the paper bag from the male plant and sprinkling it onto the silks of the female plant. Methodology has been developed to ensure that unwanted pollen does not get onto the silks. An experienced breeder can make several hundred pollinations per day.

The type of cultivar grown by farmers is influenced directly by the crop's reproductive biology. For example, in soybeans farmers grow inbred lines¹ (or varieties as they are commonly called) and in corn farmers grow hybrids. Hybrids in corn are produced by crossing two inbred lines. Hybrids and inbred lines are both homogeneous, meaning that every plant of the same hybrid or inbred line is genetically identical. Hybrids are produced in seed production fields common throughout the Midwest where it is common to see many teenagers employed to detassel the female inbred line so that only pollen from the male inbred line is used to produce the seed.

Genetic Contributions to Increases in Corn Yields

Corn breeders have been extremely successful at increasing the grain yield of hybrids. The increase in grain yield of U.S. hybrids from 1866 to 2004 is shown in Figure 2. Prior to the introduction of hybrids

¹ An inbred (or pure) line is a strain that breeds true to type when individual plants of the strain pollinate themselves (when a plant pollinates itself, it is called self-pollination).

in the 1930s, average U.S. grain yields changed very little. The lack of change may be due to a variety of factors, including lack of the management and cultural practices to maximize the performance of the genetics being grown by farmers. The introduction of hybrids in the 1930s coincided with the introduction of modern fertility and management practices (Russell, 1991). Since the introduction of hybrids, corn yields have increased from an average of about 30 bushels per acre to an average of about 150 bushels per acre — a five-fold increase in 75 years. Corn breeders are interested in how much of this increase is due to genetics and how much can be attributed to other factors. Recent studies have shown that at least 50 percent or more of the increase in corn yields since the introduction of hybrids is due to genetics (Russell, 1993).

Development of New Inbred Lines and Hybrids

Corn breeders have primarily focused on improving grain yield, but considerable effort also has been placed on desirable agronomics, such as standability, dry down, disease resistance, and drought tolerance. All these traits are what breeders would call complex or quantitative traits; that is, they are controlled by many genes. Breeders know a great deal about the general nature of these traits and how they respond to selection due to basic research at both public and private institutions and from experience in working with these traits.

The primary limitation facing breeders in selecting for any trait is our ability to define the trait and our ability to measure the trait in an inexpensive and relatively high-throughput way. The greatest success story in breeding has been the development of plot combines which give breeders the ability to quickly and inexpensively measure

grain yield, grain moisture, and test weight. Measurements taken from combines for grain yield, grain moisture, and test weight directly relate to traits farmers need. The development of plot combines is one of the important reasons for the genetic gain achieved in corn. The measurement of protein and oil content is also a success story, from the standpoint of speed and expense. The long-term selection programs at the University of Illinois have demonstrated how measurements from near infrared reflectance (NIR) technology can be used to select for high and low protein or oil content (Dudley and Lambert, 1992). Measuring protein content alone, however, does not guarantee a high quality protein with a balanced amino acid profile. To obtain corn with high protein content and a balanced amino acid profile would require a more complex selection program that measured both protein content and amino acid content.

Selecting for complex traits like yield has been the primary approach breeders have used to improve corn. Another approach is to incorporate a single gene or group of genes that gives the same trait (phenotype) in most all environments. There are two approaches to this type of breeding. One is to use native corn genes. An example of this would be the Ht gene in corn which gives resistance to northern corn leaf blight (NCLB, *Exserohilum turcicum*). Hybrids containing the Ht gene would be resistant to most, but not all races of NCLB. The second approach to using single genes is the transgenic approach. Insect resistance and herbicide resistance are examples of transgenes² that have been added to corn

² A transgene is a gene that has been engineered by molecular geneticists to have specific properties, which is then inserted into the plant by the general process known as transformation. The gene may or

hybrids. Once a transgene has been inserted into a plant it is, in effect, a single gene for the trait it confers.

Developing a corn hybrid involves developing or identifying two inbred lines that, when crossed together, produce a superior hybrid. Actual procedures for developing inbred lines vary somewhat from one breeding program to the next. All inbred development programs, however, are characterized by repeated generations of self-pollination and simultaneous evaluation of the experimental inbred in hybrid combinations for complex traits (Hallauer, 1990). It is during the inbreeding process that corn breeders determine the yield and agronomic potential of a new inbred line. Single gene traits are not incorporated into inbred lines during this phase of the breeding program.

Gene Discovery and Transformation

Transformation is the process of inserting a gene into a plant that has been engineered by molecular biologists. Transformation basically consists of three steps: (1) introduction of the gene into plant cells — this is usually done in corn using a method called particle bombardment. (2) Regeneration of a plant from cells via tissue culture; and (3) Growing the regenerated plant in the greenhouse to produce seed.

Not all corn cultivars are amenable to transformation and introduction of a gene into a cell; producing seed containing a functioning gene continues to be a difficult process with a relatively low success rate. Most transgenes are initially inserted into agronomically inferior germplasm that has been selected specifically for its ability to provide a relatively high rate of successful

may not come from other species, but many of the genes currently in use have come from bacteria.

transformation events ((Armstrong et al., 1991). Once the gene has been inserted into a plant, molecular biologists must conduct experiments to ensure the gene is inherited from one generation to the next and to be certain the gene is expressing the protein of interest. Once this step has been completed, the gene is ready to be moved into commercial cultivars.

The discovery of new genes that control traits of interest for insertion into corn is made in a variety of ways. Genes controlling some traits of interest are discovered by chance during basic molecular biology and plant physiology research. Genome sequencing projects can also lead to the discovery of interesting genes for insertion into corn. In corn, there is a Maize Gene Discovery Project that uses multiple molecular biology approaches to discover genes of interest to scientists ((Lunde et al., 2003). It is important to note that transgenes are not always genes from other species.

Integrating New Traits into Hybrids

Because the germplasm from which plants are regenerated following the insertion of a gene is not commercially viable, a backcrossing procedure is initiated immediately after plant regeneration to move the gene of interest into commercially viable inbred lines.

The first step in moving the transgene into an elite inbred line is to cross the transformed plant with the elite inbred. This cross to an elite inbred line initiates a breeding procedure called backcrossing. Backcrossing is designed to move single genes from one inbred line into another inbred line. A typical backcrossing scheme is outlined in Figure 3. The inbred line that the transgene will be transferred to is called the recipient (breeders refer to this line as

the recurrent parent). The recipient inbred is an elite inbred that is already used in commercial hybrids. The inbred line or plant that will be donating the gene is called the donor. The goal of backcrossing is to transfer the gene of interest from the donor into the recipient without otherwise genetically changing the recipient.

The backcrossing process starts by making a cross between the recipient and donor. The plants (progeny) produced from this first generation and later generations are repeatedly crossed with the recipient inbred line. Each round of these repeated crosses is called a backcross. With each generation of backcrossing, the amount of donor genome remaining is reduced by ½. The number of backcrosses depends on many factors, but varies from two-seven with an average of about five backcrosses. The time it takes to complete the backcrossing procedure depends on the availability of off-season nurseries and whether molecular markers are used to aid the backcrossing process.

After backcrossing is completed, the newly developed transgenic inbred line is crossed with another inbred to produce a hybrid to be sold to farmers.

The important point to note about this procedure is that the transformation procedure is only conducted once. The gene is moved (backcrosses) into inbred lines by using conventional breeding methodology. Because transgenes are genetically dominant, only one inbred line of a hybrid needs to contain the transgene for it to be expressed in the hybrid.

What does the Future Hold?

Table 1 contains a list of the corn biotechnology products that were available for the 2004 growing season taken from the

National Corn Growers Association (<http://www.ncga.com>) Web site. What is interesting is that all the events currently on the market are related to either herbicide tolerance or insect resistance (both are commonly known as input traits).

A survey of petitions for deregulation for corn, which is an indication of products that may be close to commercialization contained a total of 20 events that have been approved for deregulation or are pending a decision on deregulation. Of these 20 events, only one is for an output trait: high lysine content. The remaining 19 were mostly herbicide tolerance or insect resistance or a combination of the two. It would appear that output traits are still some time from being a reality in farmers' fields.

There are, however, a large variety of output traits and bio-pharmaceutical traits that are in some phase of field testing. How many, if any, of these make it to market is difficult to predict.

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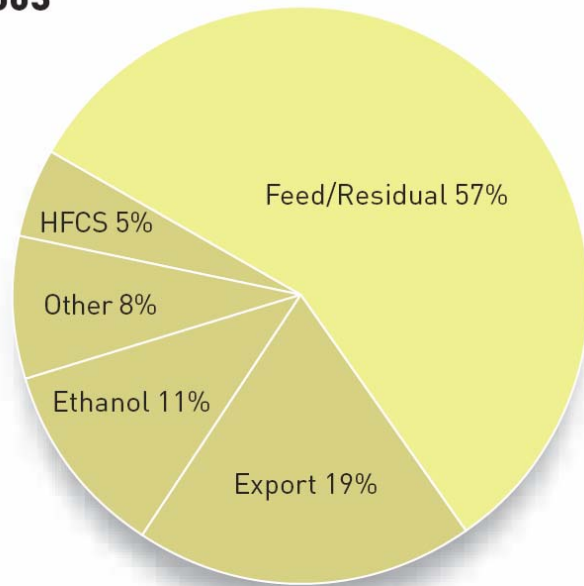
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U.S. Corn Usage by Segment, 2003*

Million Bushels

Feed/Residual	5,775
Export	1,975
Food, Seed, Industrial	
Ethanol	1,120
HFCS	535
Starch	260
Sweeteners	225
Cereal/Other	188
Alcohol	132
Seed	20
Total Use	10,230



*Marketing Year ending Aug 31, '04 - Source: USDA, ERS, Feed Outlook, Jan '04

Figure 1. Corn usage data for 2003. Figure taken from the World of Corn 2004 Web site sponsored by the National Corn Growers Association (<http://www.ncga.com/WorldOfCorn/main/consumptionData.htm>).

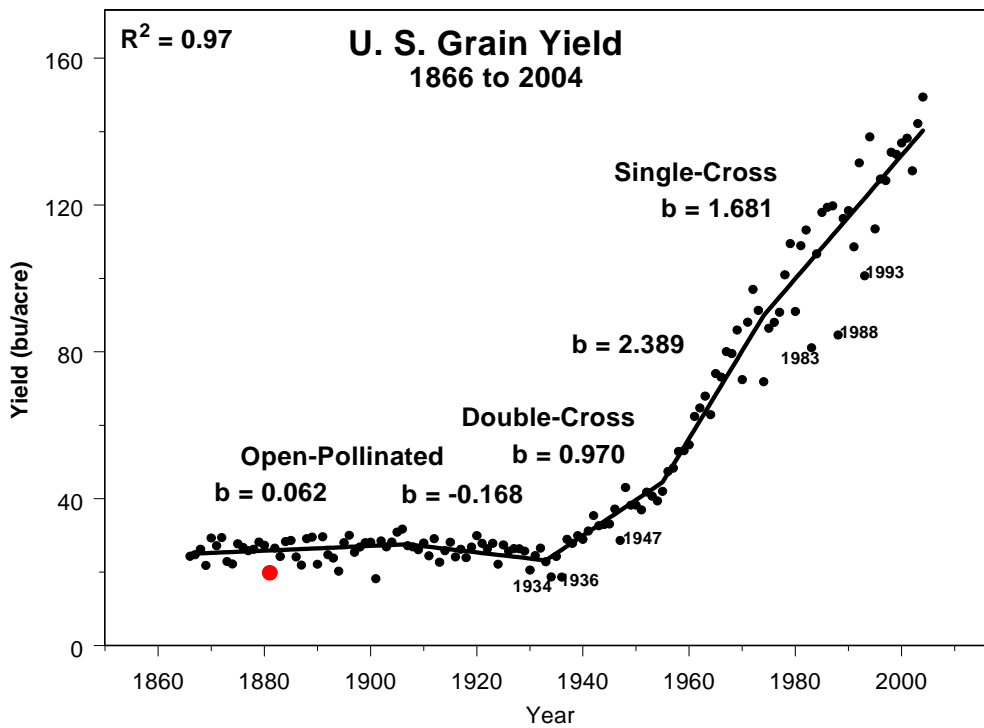


Figure 2. Plot of U.S. grain yield for corn from 1866 to 2004 (August 1, 2004 estimate). The b-values are the average rates of change in grain yield in bushels per acre per year for the particular line segment. Data were obtained from the USDA-National Agricultural Statistics Service (<http://www.usda.gov/nass/>).

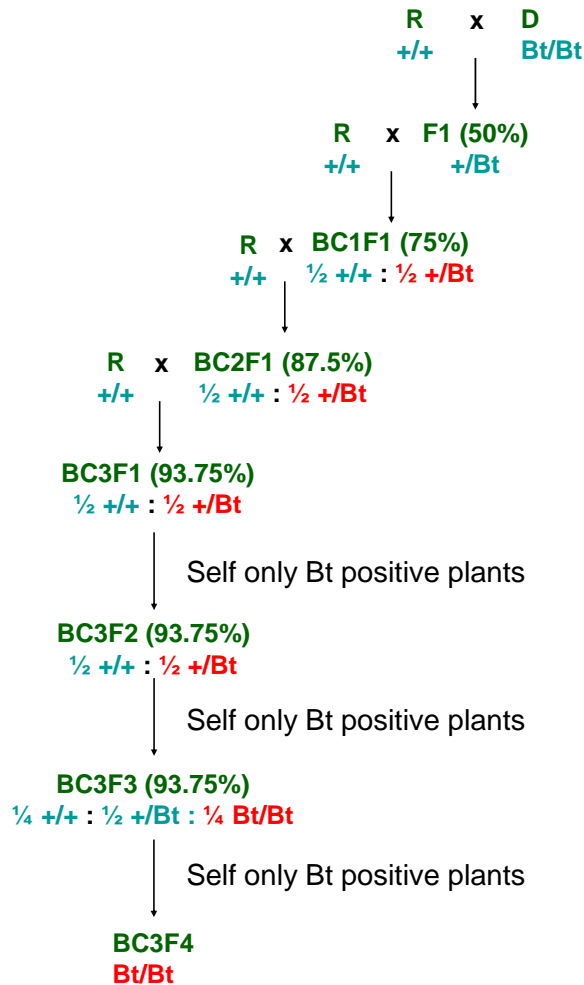


Figure 3. A schematic outline of a backcrossing program in plants. In this example, a gene called Bt is being backcrossed from a donor inbred (D) into a recipient inbred line called the recurrent parent (R). The genetic contribution of each generation is listed under the generation. The +/+ genotype is wild-type and the Bt/Bt genotype is homozygous for Bt. BC followed by a number is the backcross generation number. F followed by a number is filial generation. For example, BC1F₁ is the backcross generation 1 and the F₁ means it is the first generation cross. BC3F₂, means the backcross 3 F₁ has been selfed once.

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EVENT	CHARACTERISTICS	PRODUCT REGISTRANT TRADE NAMES	U.S. APPROVAL	JAPAN APPROVAL	EU APPROVAL
Bt11	Corn borer resistance Glufosinate herbicide tolerance	Syngenta YieldGard, Liberty Link	Yes	Yes	Yes
Bt176	Cry1Ab corn borer resistance	Syngenta Knockout, Mycogen NatureGuard	Yes	Yes	Yes
TC1507	Corn borer, black cutworm and armyworm resistance Glufosinate herbicide tolerance	DowAgrosciences Pioneer Hi-Bred Herculex I	Yes	Yes	No
Mon810	Cry1Ab corn borer resistance	DEKALB YieldGard	Yes	Yes	Yes
Mon810+GA21	Cry1Ab corn borer resistance Glyphosate Herbicide Tolerance	DEKALB YieldGard Roundup Ready	Yes	Yes	No
Mon810+Nk603	Cry1Ab corn borer resistance Glyphosate Herbicide Tolerance	DEKALB YieldGard Roundup Ready	Yes	Yes	No
Mon810+T25	Cry1Ab corn borer resistance Glufosinate herbicide tolerance	DEKALB YieldGard, Aventis Liberty Link	Yes	Yes	No
Mon863+GA21	Corn Rootworm Protection Glyphosate Herbicide Tolerance	YieldGard Corn Rootworm Control, Roundup Ready	Yes	Yes	No
Mon 863+Nk603	Corn Rootworm Protection Glyphosate Herbicide Tolerance	YieldGard Corn Rootworm Control, Roundup Ready	Yes	Yes	No
Mon863	Corn Rootworm Protection	YieldGard Corn Rootworm Control	Yes	Yes	No
MonGA21	Glyphosate Herbicide Tolerance	DEKALB Roundup Ready	Yes	Yes	No
Nk603	Glyphosate Herbicide Tolerance	DEKALB Roundup Ready	Yes	Yes	No
T25	Glufosinate herbicide tolerance	Aventis Liberty Link	Yes	Yes	Yes

Table 1. A list of biotech seed products available for the 2004 planting season. This list was taken from the National Corn Growers Association Web site on biotechnology (http://lepton.marz.com/ncga/search_hybrids/know_where.asp). The list may not contain all corn biotechnology products currently available.