

CURRENT AND FUTURE GENETICS IN CORN SILAGE

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ABSTRACT

Attaining high-quality corn silage is a critical aspect of profitability for corn and animal productivity. Biotechnology traits that reduce stress likely have more potential yield benefits for silage growers than even grain farmers. The corn genomes have been sequenced. We've put in thousands of markers that let us tag individual genes. Breeders have tools unlike anything they've had in the history of plant breeding. These genetic advancements coupled with trait and agronomic improvements across the industry are quickly changing the way corn grown for silage performs.

Key Words: corn silage, biotechnology, breeding tools, genetic advancements

INTRODUCTION

Corn silage is a popular feed with dairies because the corn plant can provide large volumes of digestible, palatable feed and requires harvesting only once-a-year while possessing the ability to utilize large quantities of manure. The seed that produces corn silage is no longer just a seed. It is a whole package. Tomorrow's trait pipeline contains unique proteins capable of fending off more species of insects, potential for high-yield genes, and more efficient nitrogen use. Biotechnology has certainly been an important instrument in maintaining the historical legacy of continuous improvement in the agronomics and yield of corn. This paper provides an overview of the yield, traits, nutritional advances in corn genetics and breeding.

CORN SILAGE IN THE MARKETPLACE

Com silage is a high-yielding and high-quality feed commonly fed to dairy cows. Com silage is produced by harvesting whole com plants a few weeks before physiological maturity and ensiling. The most significant carbohydrate sources in com silage are from the starch and cell wall fractions, which are made available to the animal by a complex community of anaerobic microbes that reside in the gastrointestinal tract. Both highly degradable (starch and simple sugars) and less degradable (cell wall bound) carbohydrates are converted to volatile fatty acids and absorbed through the rumen wall. (Van Soest, P. J. (1994) Metabolizable energy that exceeds maintenance requirements in a healthy, lactating dairy cow is used for milk production.

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CORN SILAGE BREEDING

Plant breeding is the art and science of changing the genetics of plants for the benefit of mankind. Plant breeding can be accomplished through many different techniques ranging from simply selecting plants with desirable characteristics for propagation to more complex molecular techniques. These include the use of Doubled haploids, transformation, genomics and molecular markers. These technologies have allowed scientists to make more informed decisions around specific genetic combinations to improve genetic gain, and allowed for more rapid identification of lines carrying a particular trait of interest. Utilization of conventional breeding coupled with biotechnology has led to the rapid increase in both quality and quantity per acre of the corn silage grown.

Conventional Breeding

Traditional corn breeding programs in the developed world breed hybrid varieties for farmers' use. Hybrids in the United States today are mostly crosses between two inbred lines. New inbred lines are developed by selfing plants from a source population. Source populations could include open pollinated varieties, synthetics, or crosses between two or more inbred lines. Successful commercial corn breeding programs today often start with source populations created by crossing two elite inbred lines that both combine well with another line (tester) to produce hybrids exhibiting improved levels of yield. The source population is then self-pollinated for seven to eight generations, with several hundred selfed families being selected and advanced during each selfed generation. After one to three selfed generations, the selfed families are crossed onto an inbred of a complementary heterotic group (tester) and the hybrid progeny are evaluated in replicated trials for yield and desirable agronomic traits. Lines from the selfed families that produce the best tester hybrids are advanced to further selfing generations and re-crossed onto additional testers to produce new hybrids to evaluate. As the families are selfed, each generation becomes more and more homozygous, or inbred, eventually giving rise to new inbred lines. New inbred lines that produce new hybrids better than the best current hybrids are advanced. New hybrids are evaluated over several hundred locations over two to three years before a selected few are released as new commercial hybrids. (Gracen V. 2007)

Doubled Haploids

One example advanced breeding is di haploid breeding. Dihaploid breeding allows breeders to develop inbreds in about one third of the time as conventional breeding. It still requires multiple years of testing. The time to commercialize hybrids can be reduced since the testing is done with the same hybrid from the first year.

Haploids are created by a special genetic process and have one set of chromosomes. They undergo chromosome doubling through a chemical process that produces a completely homozygous, fertile doubled haploid plant. Conventional inbred development takes a minimum of seven generations of self pollinating to get close to 100 percent pure. The purity and genetic uniformity of doubled haploid lines make it easier to measure characteristics.

Transformation

Transgenic maize for commercial production currently confers either insect resistance or herbicide tolerance or a combination of these traits. The introduction of transgenic maize has resulted in an increase in maize production. The economic impact of transgenic maize in the

global market has been tremendous because some countries have set tolerance levels set for content of transgenic maize. This tool provides scientists with the ability to improve products that cannot be accomplished with conventional breeding. In transformation, genes available from other sources can be integrated into corn to make marked improvements for a key trait.

Genomics

Genomics is the study of the DNA sequences, including structural genes, regulatory sequences, and noncoding DNA segments, in the chromosomes of an organism. Understanding the genes that influence desirable traits has the potential to increase the efficiency of selection. Recent advances in molecular biology have made it possible to acquire extensive knowledge of the genome of plants. (Gracen V. 2007) This tool helps scientists identify which genes determine important traits in corn, and how genes interact with each other. For example, gene interaction likely determines how drought tolerant a hybrid is in a particular environment. This is due to multiple genes controlling different plant functions during the stress period. Genomics help sort out the interaction. (Curran, 2008).

Molecular Markers and Breeding

Molecular marker assisted selection (MMAS) is a complementary technology that is often used in conjunction with conventional methods of genetic selection. This technology relies on the visualization of small DNA fragments that are either genetically linked to the expression of a trait, or are the genes themselves. Once a fingerprint or gene has been identified, a plant breeder can use molecular techniques to select only those plants that possess the desirable allele of that gene. Although single gene controlled traits have received most attention, progress has also been made using MMAS for selection of multiple gene traits. (M. Paul Scott, 2005)

Gene Sequencing

Gene sequencing technology is used by researchers to determine the nucleotide sequence of genes or regions of the genome important for trait improvement. This technology has also been used to create a dense genetic map that helps us understand precisely where a specific gene or trait is located, the amount of genetic diversity that exists for that specific gene or trait, and to develop DNA-based diagnostic markers for the trait for use in the breeding programs.

Gene Regulation – the ability to **Fine-tune** native gene expression.

Gene Targeting – the ability to precisely **Edit** native genes.

Trait Stacking – the ability to **Add** traits precisely where we want them.

EXZACT™ (exclusive technology of Dow AgroSciences) is the only technology capable of specifically targeting any DNA sequence. Zinc finger technology enables two powerful capabilities for crop improvement. Gene targeting is the ability to precisely edit a given gene sequence while gene regulation allows for genes to be specifically turned up or down, on or off.

Testing

Testing is a principle component of any product development program. As new lines reach the second or third selfed generation, they are crossed onto one or more tester lines to generate hybrids for evaluation. In first stage of hybrid testing, hybrids are evaluated at a low number of locations, often in 2-row plots. In stage two of testing, the best 5% – 15% of these hybrids are selected to advance and tested in often in 2- or 4- row plots. These hybrids are tested at 2-4 times the number of location as the first stage. Subsequently, in stage three, the best of stage two

hybrids are advanced to most often 4-row plots at many locations. The 4th stage is similar to the third stage. This selection of hybrids better than current, increased testing locations and in commercial and harvested using current farming practices; this comprises stages three and four of testing. In the 5th and 6th year of testing the plot size is often increased. The selection criteria remains the same Experimental hybrids must perform better than the current commercial hybrids. After six years of small-plot and large-plot testing at up to several hundred locations per year, the best-performing hybrids are approved for sale. Yield trial testing and selection is the process which identifies genetic improvement. It provides the determination of genotype X environment interaction that will allow the selection of superior inbreds.

UNIQUE GERMPLASM

North American corn genetics consists primarily of dent rather than flint background. Dent corn contains more soft, floury endosperm (hence the “dent” at the top of the kernel when it dries), which is more “open” in structure and opaque in appearance. Dent corn has various proportions of soft, floury starch to hard, vitreous endosperm and depends on the genetics. Flint corn is similar to popcorn with much more vitreous starch. European, shorter-season (<90 day) corn still contains considerable flint influence because of the agronomic advantages, such as early growth vigor, provided in flint lines. Wisconsin work showed vitreousness of flinty hybrids averaging 73%, while mature dent hybrids averaged 48% hard starch (Correa, 2002).

Laurer *et al.* (2001) reported that since 1930 forage quality in northern corn belt hybrids has improved at the rate of 3.0 kg Mg⁻¹ forage y⁻¹, and when combined with yield, has increased by 156 kg milk ha⁻¹ y⁻¹. These gains in nutritional quality and productivity were mostly unintentional and were based mostly on increased grain yield because there have been no significant silage breeding programs in the US until recently. The GEM evaluations reported show that there is tremendous potential for accelerating improvement in silage quality by using selection procedures that directly utilize nutritional components in germplasm hitherto ignored by maize breeders.

In recent years much more emphasis has been put on crossing exotic tropical germplasm with temperant germplasm from Europe, Canada, and the United States. This approach has been bringing greater genetic diversity to silage breeding programs.

Plant Modifications

Plant modifications have been made through traditional breeding and/or biotechnology to accomplish some of these goals toward ideal corn silage. High-quality corn silage hybrids have been obtained through genetic selection for improved stover digestibility (stalk, leaves, husk and cob) and grain yield. Fiber digestibility and grain yield have been research areas of interest for many years because the two are not highly related (Argillier *et al.*, 1995; Hunt *et al.*, 1992).

There are many different types of commercial hybrids developed through conventional breeding to consider for use as silage for cattle such as waxy, Opaque 2 (high lysine), brown midrib (bmr), high oil, multileaf (leafy) and high NDF. Below is a review of some of the types.

Brown Midrib: The brown midrib trait was first reported in dent corn at St. Paul, MN in 1924. It first appears at the 4-6 leaf stage as a reddish-brown coloration down the leaf midrib. The color is associated with lignified rind and vascular bundles. Coloring eventually disappears on the leaves, but remains in the stalk (Lauer and Coors, 1997). Since its initial

discovery four brown midrib mutants have been identified which include *bm1*, *bm2*, *bm3*, and *bm4*. Brown midrib corn is an example of a natural mutation in one of the lignin biosynthetic enzymes.

One bmr mutant, *bm3*, consistently decreases the lignin content of the corn plant by approximately 40%. In the presence of homozygous *bm3* in-vitro NDFD is increased (Barriere and Argillier, 1993). Of the available corn hybrid types, homozygous *bm3* is the only one that shows an improvement in milk production of 4-5 lbs/cow/day. (Oba and Allen, 1999; Bal et al., 2000; Moreilra et al., 2000; Ballard et al., 2001). Dairies utilizing commercial *bm3* hybrids have reported milk increases over 10lbs/cow/day.

Multi-leaf (Leafy): The leaf gene was first identified in the 70's. Since then, corn hybrids have been developed and commercialized with two to 10 extra leaves above the ear as compared to other hybrids of the same maturity. Having extra leaves above the ear can result in increased forage digestibility if there is increased leaf to stem ratio. Growing environment, maturity, and height of the cutter bar at harvest, can alter stover composition considerably and thus affect fiber digestibility and performance in animals. Thomas et al. (2001) and Clark et al. (2002) reported significant increases in milk production when multi-leaf hybrids were fed as corn silage. However, several researchers have reported small or no differences in milk production when lactating cows were fed leafy silages (Kuehn et al., 1999; Bal et al., 2000; Moreira et al., 2000; Ballard et al., 2001; Nennich et al., 2003). Kuehn et al. (1999) fed lactating dairy cows a diet containing 40% CS (multi-leaf, high-yielding grain, and a blend of hybrids) on a dry matter basis for the first 22 weeks of lactation. Silage yields were 5.5, 6.0, and 5.8 tonnes of DM/acre for the grain, leafy and blend silage, respectively. In vitro digestible dry matter (66.8, 69.2, and 66.7% for grain, leafy and blend silages, respectively) and digestible NDF (34.6, 38.0, and 34.4% of DM for grain, leafy and blend silages, respectively) were measured. No significant corn silage effect on cow performance was reported. Nennich et al. (2003) fed leafy hybrid as compared to conventional corn hybrids to lactating cows for 120 days (40% of diet dry matter as corn silage) and observed no differences in DMI, NDF intake, milk yield or composition.

TRAITS

Biotech – Agronomic traits: Using the tools of biotechnology insect-protection and herbicide-tolerant traits have been incorporated into corn. The insect protection traits have resulted in protection against the European corn borer, Corn rootworm and other pests. The insecticidal proteins used in this products have been found to be safe with no adverse effects when fed to dairy cattle (Hartnell et al., 2001; Barrière et al., 2001; Folmeret al., 2002; Donkin et al., 2003; Grant et al., 2003; Clark and Ipharraguerre, 2004). Benefits from the use of these traits include higher yields; increased tonnage due to less insect damage and less lodging; decreased mycotoxin (primarily fumonisin); reduced pesticidal use, exposure to humans, and the environment; and reduced grower costs. Corn containing herbicide-tolerant traits have also been shown to be safe with no adverse effects when fed to dairy cattle (Donkin et al., 2003; Grant et al., 2003; Ipharraguerre et al., 2003). Benefits from the use of herbicide-tolerant traits include the use of a more environmentally friendly production system for corn through the utilization of the no-till system; increased tonnage as a result of less competition for

nutrients from weeds; reduced herbicide costs; a more simplified weed control program; and reduced grower costs.

Agronomic traits under development or future possibilities for development in corn for silage include: drought tolerance for improved biomass production when water is limited or there is insufficient water at key times such as pollination and kernel fill; cold tolerance for improved germination in colder soil temperatures allowing for earlier planting; insect-stalk snap resistant for less field loss; increased grain to stover ratio; slower grain dry-down (this isn't as important now with the use of kernel processors on the harvester equipment); and mycotoxin resistance (primarily aflatoxin). The traits are primarily driven by the desire to improve grain production, which also includes higher forage yields per acre.

Biotech – Forage enhanced traits: Biotech tools are available to up-regulate, down-regulate or knock-out certain key enzymes in a metabolic pathway, insert new pathways, etc. through genetic manipulation. However, every metabolic alteration has consequences that need to be understood. If carbon is diverted towards the production of more starch, then there is less carbon for oil and protein production. An understanding of the key metabolic pathways in corn and the genetic components that control and influence them will be crucial in developing improved corn silages.

Using the tools of biotechnology it may be possible to reduce or alter lignin for enhanced fiber digestibility; alter carbohydrates for improved microbial efficiency in the rumen and reduce its impact on fiber digestibility and ruminal pH; increase protein content, quality and amino acid balance; enhance digestible biomass or milk production potential per acre and per ton; incorporate rate limiting digestive enzymes in the corn plant; and produce fermentation adjuvants in the plant that aid fermentation in the silo as well as in the rumen. The key is to identify those targets that will have the biggest economic impact to the dairy enterprise without sacrificing any of the key agronomic traits.

Stacked Traits

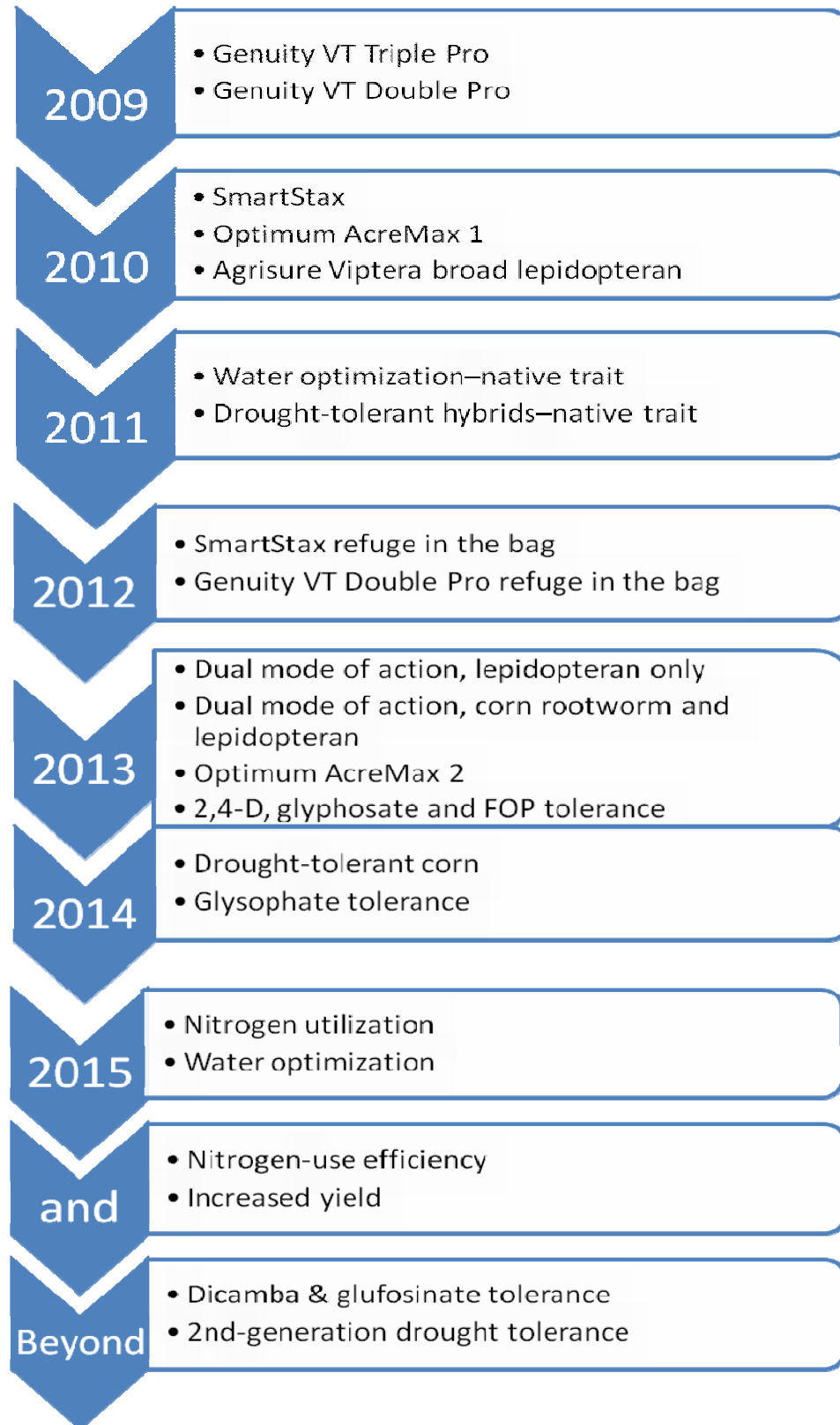
Multiply transgenic traits either herbicide tolerance, insect tolerance or a variety of combinations of the two in a single genome are what are called “stacked traits”. According to the USDA National Agronomic Statistical Service, farmers planted 70% of all their corn acres in 2010 to herbicide tolerant hybrids. Meaning those crops could tolerate applications of a nonselective, over-the-top herbicide, either glyphosate (Roundup), glufosinate (Ignite) or a combination of both. In addition 63% of all hybrids planted this year contained proteins from the soil bacteria *Bacillus thuringiensis* (Bt) for insect protection. One category protects from rootworm species that are belowground. The other provides resistance to aboveground, moth-type insects such as European corn bore. (Brooks, R., 2010)

Refuge Requirements

With all the use of Biotech insect traits comes the need for growers to be in compliance with refuge acres. In the past all you had to know was that the minimum refuge size needed to be 20% of the total corn acreage, placed appropriately. That remains for corn hybrids with Bt traits for rootworm or corn borer only. The 2010 introduction of Smartstax corn brought a structured but reduced refuge of 5% of total corn acreage. Seed technology providers (Dow AgroSciences, DuPont, Monsanto Company and Syngenta Seeds) are petitioning EPA for further approvals that

would allow blending of the refuge into the bag, but government approvals have not yet been obtained. (Smith, P., 2010)

Pipeline of Traits



Data Source for Pipeline of Traits table: (Smith, P., 2010)

CONCLUSION

This review provides important timely information about the impact of the current and future genetic impact for corn silage. We are in a world that is in constant evolution and change. Corn breeding has evolved and will continue to evolve to increase efficiency of selection to adjust to new demands and to implement sustainable production systems. Corn breeders will continue to develop, identify and find combinations of breeding systems that will maximize efficiency and improve genetic gain for the future.

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