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BREEDING FOR GRAIN QUALITY TRAITS

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ABSTRACT - Plant breeders have been extremely successful at improving the yield of maize. Grain quality has received less attention; however important advances have been made by breeders in this area as well. Maize with a wide range of compositions and fractions within the major grain components has resulted from breeders taking advantage of advances in biochemistry and genetics over the last fifty years. Breeding for grain quality provides end users with grain better suited to their needs. Maize with improved amino acid balance allows animal feed to be produced at a lower cost. Maize with altered fatty acid composition allows production of healthier vegetable oil. Maize with altered starch properties allows improvement of many products that rely on starch based gels, films and adhesives. Although mutants that impact these traits have been widely used, quantitative genetic approaches have also been successful when applied in long-term breeding programs. Two successful approaches involve elements of both approaches, including the development of QPM based on the *o2* mutant with selection for improved kernel types and the development of high amylose maize based on the *ae* mutation with selection for increased amylose.

KEY WORDS: Amino acid balance; Fatty acid content; Amylose; Amylopectin; Mutants; Starch quality.

INTRODUCTION

Breeding for grain quality has become economically beneficial because the development of niche markets for specialty grains and increasing numbers

of vertically integrated grain utilization systems can capture the added value in an improved quality product.

The feasibility of breeding for grain quality in maize is best illustrated by the Illinois Long-Term Selection experiment for protein and oil. This experiment has been running for over a century (HOPKINS, 1899, reviewed in DUDLEY and LAMBERT, 2004), and has resulted in germplasm representing the extremes of protein and oil content known in maize. In addition, this material has provided valuable insights into the physiology of metabolism in maize (BELOW *et al.*, 2004).

The focus of this review is on breeding for quality traits that are determined by the constituents of three major components of grain (protein, oil, and starch) including amino acid content, fatty acid content, and starch quality.

AMINO ACID CONTENT

Why amino acid content is important and breeding objectives

The majority of maize is used for animal feed. Ruminant livestock depend on microbial populations in the rumen to make dietary protein available for metabolism. This affords ruminants flexibility in the amino acid composition of their diets. In contrast, monogastric animals including humans require certain amino acids, termed essential amino acids, in their diets. Maize is deficient in lysine, methionine and tryptophan relative to the dietary needs of these organisms. Deficiencies in these amino acids result in poor utilization of maize protein, and nitrogen that is not used is excreted as waste. In animal feed, these deficiencies are corrected by dietary supplementation with other protein sources or synthetic amino acids, but this adds to the cost of the diet. Thus, an impor-

Abbreviations: CIMMYT, International Wheat and Maize Improvement Center; DSC, Differential Scanning Calorimetry; GEM, Germplasm Enhancement of Maize project; NIR, Near-infrared reflectance spectroscopy; NITS, Near-infrared transmittance spectroscopy; QPM, Quality Protein Maize

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tant goal for improving maize for monogastric animals is to increase the level of the limiting amino acids, while for ruminant animals, increasing the quantity of protein available is more important.

The major seed storage proteins of maize grain belong to the prolamin family and are called the zeins. These proteins make up 40-60% of the total endosperm protein and are so abundant that their properties have a large impact on the properties of maize grain protein as a whole. Zeins lack the essential amino acids lysine and tryptophan, and this deficiency is reflected in the amino acid balance of the grain. Thus, plant breeding strategies for improving the amino acid balance often involve some type of modification of the zein content.

Quantitative genetic approaches to improving amino acid content

Quantitative genetic approaches treat amino acid levels as a multigenic trait with continuous variation, and incremental improvements are made by recurrent selection. This is analogous to the way that total protein was manipulated in the Illinois Long-term Selection experiment (DUDLEY and LAMBERT, 2004). One approach to improving levels of essential amino acids is to select for total protein content. Not surprisingly, a positive correlation exists between essential amino acids and total protein content (MILLER *et al.*, 1950); however, the correlation is greater between tryptophan and non-zein protein than between tryptophan and total protein (FREY *et al.*, 1949). Consistent with these observations, several studies have found that the levels of one or more essential amino acids are negatively correlated with total protein content when expressed as a percentage of the total protein (FLYNN *et al.*, 1954; TELLO *et al.*, 1965). An additional problem with this approach is illustrated in the Illinois Long Term Selection populations in which a high protein population yielded less grain per land area than did a low protein population (BELOW *et al.*, 2004). This exemplifies a well known correlation between protein content and grain yield. While nitrogen fertilization is an effective way to increase grain yield of the high protein varieties (BELOW *et al.*, 2004), increasing total protein remains a costly approach to increasing essential amino acid levels.

Selection for the levels of specific amino acids and thereby an increase essential amino acid content without increasing total protein content proportionally should be possible. Physiologically, this could occur by reallocation of nitrogen from non-essential

amino acids into essential amino acids and would probably involve increasing the ratio of non-zein to zein proteins. This is an attractive prospect because it would not require increased nitrogen fixation or require increased inputs or decreased yield. This approach should be feasible because the level of essential amino acids has been demonstrated to be a heritable trait (DOTY *et al.*, 1946). A strategy to improve protein quality by reducing the level of zeins while increasing the concentration of tryptophan and lysine was proposed by FREY *et al.* (1949). The feasibility of this approach was demonstrated with one cycle of selection for tryptophan content which resulted in an increase in the tryptophan content of the population (FREY *et al.*, 1949). Similarly, two cycles of selection for lysine content were conducted in three maize populations resulting in improvements to lysine content accompanied by much smaller changes in protein content (ZUBER and HELM, 1972). A third cycle of selection was conducted and all cycles were evaluated in the same environment. Two of the three populations studied had increased lysine levels and total protein content following each cycle of selection (ZUBER, 1975). Lysine content was reported to have a relatively high heritability, and high lysine levels could be maintained in subsequent generations of breeding (CHOE *et al.*, 1976).

While recurrent selection to increase amino acid levels has been shown to be successful, there are at least two impediments to recurrent selection for increasing amino acid levels: the lack of inexpensive, high-throughput assays and the time investment required to generate substantial improvements with multiple cycles of selection.

Use of mutants in breeding for amino acid content

The poor amino acid balance of maize protein is a direct consequence of the amino acid composition of the major seed storage proteins, the zeins. Several mutants exist in which zein content is altered and therefore the amino acid balance of the kernel is also altered. Kernels carrying the mutation, *opaque2*, have elevated levels of the essential amino acids lysine and tryptophan due to a reduced content of zeins (MERTZ *et al.*, 1964). From the time of this discovery to the present, *opaque2* and other mutants that improve the amino acid balance such as *floury2* have been used extensively in breeding programs. However, kernels carrying these mutations tend to have a number of pleiotropic effects that reduce their agronomic adaptability. They tend to be soft

(LOESCH *et al.*, 1977), which makes them susceptible to mechanical damage (LAMBERT *et al.*, 1969). In addition, mutant plants have poor germination (LOESCH *et al.*, 1978) and reduced grain yield (LAMBERT *et al.*, 1969; SREERAMULU and BAUMAN, 1970). Thus, much of the effort in breeding programs based on these mutants is devoted to overcoming these adverse pleiotropic effects. In a thirty year effort, breeders at CIMMYT (the International Wheat and Maize Improvement Center) have successfully done this to produce Quality Protein Maize (QPM). QPM varieties carry the *opaque2* mutation and have elevated lysine and tryptophan levels. However, the kernels are harder than normal *opaque2* types and therefore much better adapted agronomically. The development and characterization of QPM are based on a tremendous effort by many researchers at the University of Illinois, Purdue University, the Istituto Sperimentale per la Cerealicoltura, and other institutions, in addition to CIMMYT, and have been thoroughly reviewed (BJARNASON and VASAL, 1992; PRASANNA *et al.*, 2001; VASAL, 2001). The development of QPM is clearly one of the most important advances in breeding for grain quality. Accordingly, the 2000 World Food Prize was awarded to Dr. Surinder Vasal and Dr. Evangelina Villegas for their roles in developing QPM. It is estimated that QPM was produced on more than 1 million hectares in 2003.

A high methionine line of maize was identified by screening for seedlings resistant to levels of lysine plus threonine that are normally inhibitory to growth (PHILLIPS *et al.*, 1981). This line was designated BSSS53 and was later released as B101 (HALLAUER and WRIGHT, 1995). The gene associated with the elevated methionine trait, *dzt 1* (originally designated *Zpr10/(22)*; BENNER *et al.*, 1989) functions by increasing RNA levels for a 10 kDa zein which is high in methionine (KIRIHARA *et al.*, 1988). BSSS53 was used as the donor parent to develop back-cross derived inbreds that were tested in hybrid combinations. Hybrids derived from the back-crossed inbreds produced 23-43% higher methionine than control hybrids (OLSEN *et al.*, 2003).

FATTY ACID CONTENT

Why fatty acid content is important and breeding objectives

Oil provides a concentrated source of energy for animals and therefore there is interest in increasing the oil content of maize grain to increase the caloric

content of the grain. In addition, oils with certain fatty acid compositions are well suited to certain end uses. Although corn oil is relatively stable to oxidative changes during storage because of its native fatty acid composition, new information about the relation of fatty acids in the diet to human health has shifted oil consumption to more highly unsaturated oils such as canola, soybean, and sunflower oils and to monounsaturated oils such as olive oil. Aggressive breeding for the fatty acid compositions of some of these oils has improved them. There is sufficient evidence to suggest that increased consumption (within limits of total fat ingested) of certain groups of fatty acids (monounsaturated and polyunsaturated) may be beneficial (NATIONAL RESEARCH COUNCIL, 1989). If corn oil is going to compete with these oils in the future, the issue of altering its fatty acid content must also be addressed.

Typical Corn Belt corn contains oil having about 10% palmitic, 2% stearic, 25% oleic, 62% linoleic and 1% linolenic acids, with a total of 12% saturated fatty acids. In a study using exotic germplasm, fatty acid composition was used as a classification criterion for Italian corn populations, and wide variability for composition was found (CAMUSSI *et al.*, 1980). Because wide ranges of fatty acid compositions can be found in adapted (DUNLAP *et al.*, 1995b) and exotic germplasm (JELLUM, 1970; DUNLAP *et al.*, 1995a), it might be possible to develop specialty corn oils, each with a unique fatty acid pattern, just as different plant species produce oils with unique compositions.

Some compositional goals and their uses are:

1. Low total saturates of 6% or less to compete with highly polyunsaturated fats such as canola and new soybean oils.
2. High total saturates to provide oil "naturally" hardened for margarine manufacturing. By minimizing processing, this oil would be low in unhealthy trans fatty acids. Researchers have produced soybean oil with these properties.
3. High oleic acid to provide an oil high in monounsaturated fatty acids to compete with canola, olive and new soybean oils.

Genetics are known to have a significantly greater impact than environment on corn oil fatty acid composition. Until recently, however, little or no effort has been made to establish gene pools that carry favorable alleles for altering levels of certain fatty acids, even though little doubt exists that corn may be bred for these traits (ALEXANDER and CREECH, 1977). Research suggests that substantial progress could be made rather quickly. Plant introductions

representing a diversity of geographic origins and agronomic characteristics were studied for fatty acid compositions by JELLUM (1970). He found extensive ranges of variation in fatty acid composition in the exotic materials that were much higher than the ranges previously reported for corn oil. JELLUM developed an inbred line (GE 180) from one of these introductions (PI 175334) that had 18-19% stearic acid, compared to 2% in normal corn oil (JELLUM, 1984). JELLUM and WIDSTROM (1983) reported that the high stearic acid trait was caused by a single major recessive gene with modifier genes in sister lines of GE 180. Agronomic characteristics of GE 180 are poor and it has not been used commercially. A discovery by ALEXANDER and JELLUM (1992) led to an international patent by The Lubrizol Corporation for corn oil with high oleic acid content (45-57%). Additional breeding produced progeny with seed containing 65% oleic acid in the oil. NAGEL (1999) developed inbred lines through mutagenesis with low saturated fatty acid content that led to an international patent by Mycogen Plant Science, Inc., WRIGHT (1995) recovered a gene from mutagenized B73 that increased the oleic acid concentration from 27 to 52%.

Other fatty acids that have been studied genetically are linoleic, oleic, palmitic, and linoleic (PONELEIT and ALEXANDER, 1965; JELLUM, 1966; PONELEIT and BAUMAN, 1970; DE LA ROCHE *et al.*, 1971; WIDSTROM and JELLUM, 1975). PONELEIT and BAUMAN (1970) determined that progress could be made in selecting for both fatty acid quantity and quality using breeding systems that exploit additive genetic variance.

The lines used in the previous studies were largely derived from adapted U.S. germplasm. DUVICK *et al.* (2003) utilized germplasm introgressed with genes from *Tripsacum*, a wild relative of corn, to alter fatty acid content in corn lines. Lines were developed that had elevated levels of oleic acid and others had either elevated or lowered saturated fatty acids. The lines with elevated saturated fatty acid composition had better oxidative stability than traditional corn oil (SHEN *et al.*, 1999).

STARCH QUALITY

Why starch quality is important and breeding objectives

Corn starch is used for many purposes including food pastes and sweeteners, moisture absorbing products, fuel ethanol production, and as a thickening agent in many products. Starch is frequently

modified chemically to improve its suitability for these uses. If starch could be modified genetically to meet these demands, the cost of products requiring modified starch would be reduced. Thus, one breeding objective is to produce starch with novel physical properties that would benefit certain end users. One of the most important physical properties of starch is its ability to form a gel when heated with water. Thus, creating starch with altered thermal properties would be a valuable modification (WHITE, 1994).

Corn starch accumulates in endosperm tissue in the form of insoluble granules. It is composed of two glucose polymers, amylopectin and amylose, which differ in their chain length and degree of branching. Amylopectin is more highly branched and normally constitutes about 75% of the starch granule, while amylose is mostly linear and constitutes 25% of the granule. These two polymers have different physical properties so much of the effort of breeding for unique starch properties have involved changing the relative proportions of these two polymers.

Industrial processes

One important breeding objective is to increase the amount of extractable starch during wet-milling. ZEHR *et al.* (1995) did a survey of wet-milling properties of 15 inbred lines and 20 related hybrids. They found, using a laboratory-scale procedure (ECKHOFF *et al.*, 1996), that gene action for starch recovery after wet-milling appeared to be additive. They also found a positive correlation between starch yield and kernel composition of starch using near-infrared reflectance spectroscopy (NIR). DIJKHUIZEN *et al.* (1998) also found positive correlations between starch composition determined by NIR and starch yield after a laboratory-scale wet-milling procedure using families derived from a cross between the Illinois High Protein and the Illinois Low Protein corn populations.

SINGH *et al.* (2001) evaluated wet-milling properties for 49 Latin American accessions used in the Germplasm Enhancement of Maize project (GEM) and found that they wet-milled poorly compared to the commercial check hybrids. In general, the exotic populations had higher protein and lower starch composition levels than the hybrids, and poor starch-gluten separation in milling led to starch with greater than acceptable protein content. In examining samples of two hybrids harvested at various stages of maturity, JENNINGS *et al.* (2002b) found that starch yield after a laboratory-scale wet-milling procedure was not affected by hybrid or maturity.

However, DIEN *et al.* (2002) found that percent of total starch available for conversion into ethanol did vary significantly among hybrids when they underwent the dry-grind ethanol process, indicating that ethanol yield is not exclusively dependent on starch composition. A study of ethanol yields in 91 commercial hybrids showed a variability of 23% in ethanol yields (SINGH *et al.*, 2004). Other factors that affected ethanol yield in their study were kernel hardness with increased density, resulting in higher ethanol yields, and planting location.

Several seed companies have classified their hybrids for highly fermentable starch using proprietary NIR calibrations. However, there has been little breeding for industrial processes in either the public or private sector (SIMON, 2004).

Waxy starch

Waxy starch refers to starch produced by varieties carrying a mutant allele of the *Waxy1* (*Wx1*) gene. The *Waxy1* gene is required for the biosynthesis of amylose, and mutant alleles of *wx1* produce *waxy* starch that is 100% amylopectin. Films made from *waxy* starch tend to be clear and *waxy* starch gels are resistant to re-crystallization. *Waxy* starch is also used in many adhesive applications.

Maize with a *wx1* mutant allele was discovered in China in the early 1900's (COLLINS, 1909), but did not become commercially important until World War II when tapioca starch, which had similar properties, became unavailable. FERGASON (1994, 2001) has reviewed the breeding of *waxy* maize, which has largely been done in the private sector. Breeding of *waxy* maize is relatively straightforward because *waxy* starch in kernels and pollen grains stains reddish-brown when treated with potassium iodide while normal starch stains blue. The back-cross breeding method has been used extensively to convert elite lines of the best commercial hybrids to the *waxy* phenotype. Because this method ensures that *waxy* hybrids will always be inferior to newer commercial hybrids, effort has been placed upon developing *waxy* breeding populations as the numbers of *waxy* converted inbreds increased. This has led to the development of *waxy* hybrids with small or insignificant yield differences compared to their non-*waxy* counterparts.

High amylose starch

In contrast to *waxy* starch gels, high amylose starch gels are opaque and have a higher strength, making high amylose starches useful in confections,

applications requiring films, and biodegradable packaging foam. VINEYARD and BEAR (1952) reported the discovery of a mutant amylose extender (*ae*) allele, which increases the ratio of amylose to amylopectin in starch, but does not change the total amount of starch in the grain.

FERGASON (1994, 2001) also reviewed the breeding of high-amylose corn, which, compared to breeding *waxy* corn, is more difficult. Mutant *ae* alleles do not confer 100% amylose starch, but rather a range of amylose percentages depending on the genetic background and environment. An unknown number of genetic modifiers interact with *ae*, contributing to the range of values of amylose found when inbred lines are converted. In addition, the relative amount of amylose in breeding lines and populations must be determined by chemical analysis, thus the breeding program must be integrated with a wet laboratory or have access to a source of accurate amylose determinations. This adds a significant expense to the breeding program. Because environment does have a major influence on amylose levels, the separation of genetic versus environmental effects is important in order to make genetic progress. In spite of these limitations, selection and breeding have increased the amylose content of high amylose hybrids from approximately 50% to well over 70% (BOYER and HANNAH, 1994).

Advances in breeding for amylose content can come from new breeding methods, cheaper and more rapid methods of analysis, or new modifier genes. CAMPBELL *et al.* (1997, 1999) investigated near-infrared transmittance spectroscopy (NITS) with calibration for amylose content. Their calibration had limited precision and thus could not replace chemical analysis, but can be used by grain handlers needing to detect severe contamination, or by breeders doing initial screening of large sample sizes. The NITS calibration did not discriminate well among genotypes with overlapping amylose contents, for example in comparison of *ae* single to *ae* double mutants (CAMPBELL *et al.*, 2000). In a study to compare NITS and a relatively rapid and inexpensive wet chemistry method with the standard iodine-binding wet chemistry method, CAMPBELL *et al.* (2002) found the relatively rapid wet chemistry method to be useful for identifying genotypes with high apparent amylose concentrations. Another important finding of this study was that exotic germplasm might be an important source of new modifying factors. Previously, ROBUTTI *et al.* (2000) had found higher amylose contents in both high

starch content and low starch content races when examining 239 accessions of 12 Argentinean landraces. The values were generally higher than found in U.S. maize.

Other mutants

The relationship between the major structural features of starches (amylose and amylopectin) and their function is fairly well understood, but effects of the fine structural characteristics on function are far more difficult to decipher. The application of differential scanning calorimetry (DSC) to starch gelatinization with improved gel permeation chromatography techniques to study starch fine structure has aided in this pursuit.

There is increasing difficulty in obtaining regulatory approval of chemically modified starches for the food industry (SANDERS *et al.*, 1990), so there is great potential for using novel starches from new corn lines. The production of corn starches with naturally occurring modifications does not require expensive processing for modifications, and so could provide additional profits over chemically modified starches. Also, because of consumer demands, there is a premium price paid by the industry for ingredients that are "natural". In some cases, the food industry may sacrifice quality to put all "natural" ingredients on the label thereby severely limiting the range of functionalities of the available natural starches.

Several known endosperm mutants have large effects on quality of corn starch (INOUCHI *et al.*, 1984; BROCKETT *et al.*, 1988; SANDERS *et al.*, 1990; WANG *et al.*, 1992, 1993a,b,c). Endosperm mutant genes also can be combined in multiple sets of two or more genes that cause genetic interactions resulting in starches with unusual characteristics (BOYER and HANNAH, 1994). These genetic interactions have been exploited commercially for high-value starches as described in several patents (WURZBURG and FERGASON, 1984; ZALLIE *et al.*, 1986; FRIEDMAN *et al.*, 1988a,b,c,d,e,f; FRIEDMAN *et al.*, 1989a,b). Production of these starches was later abandoned because of the deleterious effect of the mutants on agronomic productivity.

The *sugary-2* allele (*su2*), first described by EYSTER (1934), has many effects on starch quality including higher amylose content and lower gelatinization temperature content as measured by differential scanning calorimetry (INOUCHI *et al.*, 1991), resulting in a patent on the use of *su2* starches (WHITE *et al.*, 1994). CAMPBELL *et al.* (1994) demon-

strated a dosage effect at the *su2* locus on starch structure and function. Previous investigations showed no effect on amylose content by gene dosage associated with the *su2* allele of corn (KRAMER and WHISTLER, 1949); however, by evaluating functional characteristics such as DSC values, viscosities, gel strengths, and structural characteristics such as x-ray diffraction, a dosage effect was observed. Research examining the presence of modifying factors in exotic germplasm on starch mutant effects showed wider ranges of DSC values in two *su2* exotic populations than in *su2* with a Corn Belt background (CAMPBELL *et al.*, 1995b). Similar to the case with *ae* starch, this indicated that genetic modifiers could be used to alter thermal properties and possible functional properties of *su2* starch.

By increasing the size of starch particles, the efficiency of wet-milling may increase because less starch would be lost in the various steps in the process. In addition, particle size affects efficiency of starch modification and has a role in applications such as degradable plastic films, powders, and fat substitutes. The soft starch (*b*) gene, first described by MUMM (1929), causes the starch particles in the granule to be loosely packed. Several studies have shown that the gene also causes the granules to be larger (BROWN *et al.*, 1971; WANG *et al.*, 1993c; WILSON *et al.*, 2000a,b). WILSON *et al.* (2000a) found the trait to be predominantly controlled by additive but also by some dominance effects, suggesting that the allele could be used for developing hybrids that wet-milled more efficiently. WILSON *et al.* (2000a) along with GUTIÉRREZ *et al.* (2002), found variation in starch granule size in normal inbreds and their *b/b* conversions, while CAMPBELL *et al.* (1996) found variation in granule size in exotic germplasm.

Quantitative and environmental factors

Factors other than those introduced through mutant genes also have been shown to affect starch properties. KRUEGER *et al.* (1987) examined the effect of inbred line differences on the thermal properties of normal cornstarch. With thermal properties as an indicator of starch primary chemical structure, they found significant variations among the maize lines. CAMPBELL *et al.* (1995a) examined thermal properties of 26 adapted and exotic inbreds and also found highly significant differences, along with highly significant inbred by year interactions. They also found correlations between thermal properties and starch viscosity and gel strength, indicating that thermal

properties measured by DSC may also be predictive of other functional characteristics. This finding was supported by results of SEETHARAMAN *et al.* (2001). LI *et al.* (1994) further demonstrated wide genetic variability in the DSC thermal properties of starches from 35 tropical corn populations. WHITE *et al.* (1990) demonstrated variability in thermal behavior among five open-pollinated populations of genetically variable maize and significant differences among plants within the same population, indicating that genetic variability for thermal behavior of the starches, and likely for starch structure, may exist within populations. Other studies have shown significant variation in thermal and functional properties of starches from Argentinean land races (SEETHARAMAN *et al.*, 2001), exotic populations used in GEM (SINGH *et al.*, 2001), and breeding materials from GEM (POLLAK, 2003).

SINGH *et al.* (2001) found that gel strengths of starches recovered from GEM exotic populations were greater than that from the commercial hybrids used as checks. In another study comparing nine exotic inbreds with nine Corn Belt inbreds, however, the Corn Belt inbreds had wider ranges for most thermal traits than the exotic inbreds (POLLAK and WHITE, 1997). There seemed to be no consistent trend in comparing values of the inbreds with those of reciprocal hybrids within sets, although there was a trend toward reciprocal differences.

Several studies describe the development of inbreds from GEM exotic by adapted breeding crosses selected for unusual starch properties (Ji *et al.*, 2003a,b, 2004). The lines generally had low onset temperature of gelatinization and gelatinization over a wide temperature range, explained by greater numbers of shorter branch chains of amylopectin and different starch granule size distributions than those of normal starch. Some lines had two independent gelatinization transitions, caused by two separate types of granules within the endosperm. Quantification of gelatinization properties over generations of inbred development showed a significant genotype interaction with breeding success for these traits. Exotic background had a large influence on the inbreeding needed to genetically fix the unusual gelatinization properties, and there was a genotype by environment interaction effect.

Maturity of maize at harvest can affect starch quality. Two hybrids harvested at three stages of development showed that gelatinization started at a lower temperature and occurred at a narrower range of temperature for immature grain than for

mature grain (JENNINGS *et al.*, 2002a). Variations in DSC properties of inbred maize starches based on kernel maturity were likely due to fine structural differences during development (NG *et al.*, 1997a). CAMPBELL *et al.* (1994) examined thermal effects of starch on inbreds and their F1 progeny at four planting dates, and found some differences in thermal traits but not for amylose content or size of the starch granules. Other studies illustrating the large environmental effect on starch quality were done by NG *et al.* (1997b), KRIEGER *et al.* (1988), and Ji *et al.* (2004).

SUMMARY

The major components of maize are protein, oil and starch. Breeders have successfully manipulated these components for more than a century, resulting in maize with a wide range of compositions. Over the last fifty years, advances in biochemistry and genetics have allowed manipulation of fractions within these main grain components and these efforts have resulted in grain that is better suited to a variety of end uses. Amino acid balance, fatty acid composition and starch physical properties are important targets for modification because they impact the value of grain for animal feed, human health and industrial applications. The most widely used approach has been to use single gene mutants to give step-wise improvements in these traits. Quantitative genetic approaches have been successful when applied in long-term breeding programs. The most successful approaches involve the use of recurrent selection and mutants together, for example, the development of QPM based on the *o2* mutant with selection for improved kernel types and the development of high amylose maize based on the *ae* mutation with selection for increased amylose.

Outlook for the future

The 50th anniversary of *Maydica* comes at a time of transition in grain quality trait breeding. New targets are developing as new uses for maize are developed. The fastest growing use of maize is fuel ethanol production, which will benefit from increased levels of fermentable carbohydrates in grain. While mutants have been the basis of breeding for grain quality traits for the past 50 years, relatively few new mutants with utility in breeding programs have been identified in recent years in spite of efforts to produce new mutants by mutagenesis

(YAMIN *et al.*, 1999). Technologies are emerging to fill this niche, however. Biotechnology allows transgenic approaches that result in single-gene step-wise gains in quality traits in the same way that mutants do. Like traditional mutants, engineered genes require back-crossing to develop agronomically acceptable lines. And like traditional mutants, engineered genes may have detrimental pleiotropic effects that will have to be overcome, for example, maize engineered to have reduced zein content has opaque phenotype kernels (HUANG *et al.*, 2004). Thus, in the next 50 years, quality trait breeding will likely involve a shift from using natural or induced mutations to using transgenic approaches. Quantitative genetic approaches will remain crucial to the success of these programs, and long-term investments in recurrent selection for quality traits should be encouraged.

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