

# **The History and Success of the Public-Private Project on Germplasm Enhancement of Maize (GEM)**

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## **I. INTRODUCTION**

The Latin American Maize Project (LAMP) was the first coordinated international project for evaluating a major world crop (Salhuana et al., 1991; Pollak, 1993; Eberhart et al., 1995, Salhuana and Sevilla, 1995, Salhuana et al., 1998). In LAMP, 12 countries cooperated to evaluate their native germplasm accessions. Before the LAMP accessions could be used as sources of breeding material in the USA, some process had to be established to enhance them so

they could enter commercial corn breeding channels. A coordinated and cooperative effort among public and private sectors was organized, the Germplasm Enhancement of Maize project (GEM). The project provides the corn industry early breeding lines by using germplasm enhancement to improve and adapt useful exotic germplasm. The ultimate objective is to improve and broaden the germplasm base of corn hybrids grown by American farmers. Traits targeted for improvement are agronomic productivity, disease and insect resistance, and value-added characteristics. The project has grown to include international cooperators, both public and private.

#### **A. The Need for Maize Enhancement**

Corn is the USA's major crop (USDA-ERS, 1995) , where over 30 million hectares are planted each year. The USA is also the world leader in corn production, producing over 41% of the world's production in 1989/90 (USDA, 1990). Corn is extremely important to the U.S. economy due to the amount produced, its value to industry, and its export value. As a raw material, corn added over \$17 billion to the economy in 1989. About 20 percent of the production is exported, providing a positive contribution to the nation's trade balance (USDA, 1996). Approximately 17 percent is industrially refined. An additional \$1.4 billion in refined products is exported. Through feeding livestock that is processed into meat and dairy products, corn affects nearly everyone in American society. It has been estimated that 90% of domestic corn grain is used as food through the feeding of livestock (Hodge, 1982). Although corn is so valuable to the U.S. economy, less than one percent of the U.S. germplasm base consists of exotic germplasm (Goodman, 1985) leading to concerns about corn's genetic vulnerability.

Since the early 1960s there have been frequent and urgent warnings about corn's genetic vulnerability and the potential of exotic germplasm to decrease this vulnerability (National

Academy of Science 1972; Goodman 1990; Walsh 1981; Wilkes 1989; Eberhart, 1971; Longquist, 1974; Brown, 1975; Crossa and Gardner, 1987). These concerns have been reinforced by studies documenting a reduction in genetic variability among lines and hybrids (Smith, 1988; Darrah and Zuber, 1985). History shows that problems can occur when the genetic base of a crop becomes too narrow and changes in the environment, such as new pathogens, new insect pests, or unusual environmental stresses adversely affect the crop's productivity. For example, the southern corn leaf blight (incited by *Bipolaris maydis* (Nisikado) Shoemaker, race T) epidemic in corn showed the devastating nature of such an event (National Academy of Science, 1972). In this case, the epidemic was due to the widespread genetic uniformity of the crop which sped development of the disease to epidemic proportions.

Genetic variability is essential in plant breeding programs (Michelini and Hallauer 1993), but improvements in crops by plant breeding are usually followed by decreased genetic diversity particularly in materials that reach commercial production. Thus farmers' hybrids are increasingly genetically vulnerable and farmers face increased economic risk

Corn Belt Dent is one of the most productive races of corn in the world, and has adequate genetic variability for resistance to most common pests. For this reason, U.S. corn breeders have concentrated their effort on this race, which represents about 2% of the corn germplasm available in the world (Brown, 1975). In contrast to many other plant breeders, corn breeders have continued to focus on short-term breeding goals, largely because of the predominance of the private sector in corn breeding and its need for short-term results. This pattern has resulted in the development of a very narrow genetic base of corn produced on the farm, with many companies selling closely related hybrids (Smith, 1988). This may lead to a yield plateau, greatly increase vulnerability to pests, and makes it difficult meet new market demands. It is

prudent to develop alternate breeding populations from exotic sources. Geadelmann (1984) suggested that incorporation of exotic strains into adapted germplasm would increase the available genetic variability and give rise to additional heterotic vigor, lessening chances for a yield plateau. In the U.S. Corn Belt, exotic germplasm is usually considered to include unadapted domestic populations, and foreign temperate, tropical, and semi-tropical populations (Stuber, 1986).

It is evident that conventional corn breeding using adapted materials has been extremely successful in the USA. Forty years ago average yields in the Corn Belt were 38 bu/acre (Johnson, 1991), but 1987 average yield was nearly 120 bu/acre with yields over 240 bu/acre reported (USDA, 1988). Russell (1986) summarized 15 studies indicating that nearly 60% of recent yield gain was due to genetics. Although breeders are still making genetic gain for yield in adapted materials, there lately has been a decline in the rate of growth of cereal yield, possibly indicating a sign that breeding efforts are reaching the point of diminishing returns (Sehgal, 2000). The most recent survey of germplasm sources for the maize crop (Darrah and Zuber 1985) found that 88% of 1984 U.S. maize seed produced for 1985 planting included germplasm derived from one variety of the Corn Belt Dent race, Reid Yellow Dent. Most current hybrids are derived from a few inbred lines of Iowa Stiff Stalk Synthetic origin crossed with a few lines of primarily Lancaster Sure Crop origin. Smith (1988) used biochemical data to show that U.S. corn breeding and production was dependent primarily on four adapted lines (B73, A632, Oh43, and Mo17), or closely- related derivatives. It seems unreasonable to assume that most favorable alleles are concentrated in that sample.

Another reason for the reduced genetic base of maize breeding programs was found by Jenkins (1978) to be a greater emphasis on developing recycled lines instead of lines developed

from improved populations or synthetics. Private companies are, however, growing increasingly concerned about their narrow germplasm pools. Some companies have a germplasm-enhancement component to their breeding programs, although tough competition in the industry results in the tendency to focus on elite proprietary exotics from branch stations within a company.

Throughout the world approximately 50,000 accessions of corn exist in germplasm banks (Goodman, 1983; Ayad et al., 1980) and, until recently, many had never been evaluated for useful traits. Obstacles limiting effective use of plant genetic resources include lack of evaluation data, lack of documentation and information, poor coordination of national policies, and poor linkages between genebanks and breeders. Evaluation is important to identify potentially valuable traits in accessions, but most countries cite the lack of useful evaluation information as a major bottleneck to increasing germplasm utilization (Report on the State of the World's Plant Genetic Resources, 1996). The assumption that well-evaluated and documented germplasm collections will lead to their increased use by breeders has been stated by many (Kannenbergh, 1984; Wilkes, 1984; Plucknett et al., 1987; Smith and Duvick, 1989; Goodman, 1990; Anonymous, 1991; and Salhuana et al., 1991).

Germplasm bank accessions are at least 60 years behind currently used breeding populations for yield and standability. Genetic diversity exists in corn collections but little has been incorporated into elite breeding populations. LAMP evaluated over 12,000 accessions and the data are readily available to breeders. Resistance to using the elite LAMP accessions will decrease when they receive enough prebreeding to be attractive to commercial breeders. This prebreeding will be accomplished by GEM. Introgression of this germplasm into commercial materials will broaden the diversity of hybrids in farmers' fields. Materials from LAMP have the

potential to dramatically change the maize grown in the USA similar to the way materials from the Sorghum Conversion Program have changed grain and forage sorghum. Some of the important economic characteristics found in the germplasm released from the Conversion program and used to improve commercial sorghum hybrids are pest resistance, drought and saline tolerance, stalk strength, improved yield and yield stability, mold resistance, higher protein, a more desirable balance of amino acids, and superior food quality characteristics (Miller, 1979).

Diversity of germplasm sources will aid the development of hybrids with more favorable amino acid compositions for food and feed uses, lower protein content and higher starch yields for wet millers, and higher test weights, higher protein and harder endosperm for dry millers. Consumers will benefit by being assured of a stable food supply.

### **B. The Latin American Maize Project (LAMP)**

The Germplasm Enhancement of Maize Project (GEM) in the United States would not exist if LAMP had not come first. LAMP provided the information necessary to efficiently select germplasm bank accessions for enhancement. In this regard, LAMP served as the first step to share promising maize materials from the germplasm banks with breeders. GEM will complete the process by returning to the germplasm bank enhanced materials developed from the accessions, that can be directly used in applied breeding programs.

LAMP involved the cooperative efforts of 12 countries (Argentina, Bolivia, Brazil, Colombia, Chile, Guatemala, Mexico, Paraguay, Peru, United States, Uruguay, and Venezuela) to evaluate their native maize germplasm accessions for yield and agronomic characteristics (Salhauna et al. 1991). The funding (\$1.5 million) was donated in 1987 by the leading U.S. producer of hybrid maize, Pioneer Hi-Bred International, under the incentive of its CEO, Dr.

William Brown. Dr. Brown envisioned a collaborative effort among nations to characterize and regenerate maize accessions held in important germplasm banks of Latin America, leading to increased agricultural biodiversity. The funding was administered by the U.S. Department of Agriculture (USDA) Agricultural Research Service (ARS) who also provided administrative support. Each participating country donated in-kind support consisting of a principal investigator and technical and support staff. LAMP was the first coordinated international project to deal with the evaluation of the genetic resources of a major world crop. During five stages LAMP evaluated over 12,000 accessions (that belonged to 74% of total maize races) in locations divided into five homologous areas covering latitudes from 34°S to 41°N, longitudes from 44° to 101°W, and altitudes from 29-3300 m above sea level.

In 1991, a catalog and CD-ROM of data of 12,113 accessions evaluated in LAMP's first stage, and 2,794 selected (primarily on yield) accessions evaluated in the second stage in 59 different locations of 32 regions of the 12 countries was published (LAMP 1991). Based on that data the Principal Investigators in each country selected a total of 268 elite accessions that were crossed with the best testers of each region (Stage 3). Thirty-one testers were used for crossing with the elite accessions. Within a homologous area, Principal Investigators exchanged testcrosses among other Principal Investigators in the same homologous area, so that testcross evaluations were done in more than one country (Stage 4). Data from the testcross evaluations were published in a catalog and updated CD-ROM in 1995 (Salhuana and Sevilla 1995), and a final report published in 1997 (Salhuana et al. 1997).

In the fifth stage of LAMP, each Principal Investigator was to enhance selected germplasm to meet his or her country's breeding objectives, yet funding for only a year of small-scale enhancement was available. Enhancement of some of these selected accessions is an important

activity for each country, because it is likely that the productive accessions identified by LAMP are very different from the breeding populations used in the country and that they can add new genes for productivity. This is true even though the selected LAMP accessions may be native to a country in question, because before LAMP few countries used their native germplasm in breeding programs (Salhuana et al, 1997). In the U.S., we used restriction fragment length polymorphisms to determine relationships among six Caribbean LAMP accessions and the adapted U.S. inbred lines B73 and Mo17. It was very clear that the exotic accessions were very different from the Corn Belt lines, and that the Caribbean accessions were also very diverse from each other (Pollak and Salhuana, 1999).

## **II. GEM'S DEVELOPMENT**

### **A. Public/Private U.S. Agricultural Research**

U.S. agricultural research includes three sectors: federal programs, state programs in land-grant and other universities, and private industry. Both state and federal programs are considered the public sector. Federal programs include both intramural research (e.g. USDA-ARS) and extramural research (through State Agricultural Experiment Stations). Objectives of intramural federal research (e.g. USDA-ARS) include conducting research that addresses national and regional problems where state incentives are low but social payoffs are high, and research that involves higher-risk and longer-term problems. Germplasm preservation, evaluation, and enhancement fit into these objectives. States typically direct their agricultural research toward problems and crops important in the state. The private sector focuses intensively on commercially-oriented problems, and has little incentive to do high-risk and long-term research on germplasm enhancement (Knudson 1998).

The amount of plant breeding effort in maize is in excess of 25% of total U.S. plant breeding effort, calculated in scientific years (SY's). However, most of this effort is in the private sector. The private sector in 1994 employed 509.8 SY's, while the public sector employed 35.3 SY's (27.1 in the state universities, 8.2 in the USDA-ARS) (Frey 1996). This represents a serious constraint in human resources and expertise prevented the public sector from carrying out a major maize enhancement effort without private involvement.

Legal changes in intellectual property rights and technology transfer during the past few decades allow greater cooperation between the public and private sectors. Intellectual property rights such as patents increase incentives for the private sector to invest in crop research. Technology transfer laws facilitate cooperation between federal and private laboratories. These legal changes have led to the emergence of a research consortium type of institution, whereby private and public collaborators jointly engage in basic and applied research, jointly own products from their research, and sometimes share the profits. GEM's existence may be possible because of these changes in intellectual property and technology transfer laws (Knudson 1998).

The competitive nature of the seed industry made it unlikely that any one company would support an enhancement effort utilizing LAMP materials. Public breeders are poorly funded, there are very few of them compared to private breeders (Frey, 1996), and there are few if any grant sources for germplasm enhancement, so it was unlikely that public breeders could find the financial resources to support an enhancement effort. It was clear that a coordinated and cooperative effort among public and private sectors was needed before the LAMP materials would be used in U.S. breeding programs. It was also clear that coordination and primary funding of the effort would have to be by USDA-ARS because of the project's high-risk long-term research and national scope.

## **B. Public and Private Interaction to Organize GEM**

As LAMP was nearing its conclusion in 1992, there was concern among maize germplasm scientists that unless an organized effort was put into place to enhance the best LAMP accessions they were unlikely to be used in breeding programs. Enhancement proposals were prepared by Linda Pollak of USDA-ARS, and by Major Goodman of North Carolina State University and Randy Holley of Northrup King (now Syngenta), and sent to the American Seed Trade Association's (ASTA) Corn & Sorghum Basic Research Committee (CSBRC) for consideration at their December meeting. At the December 1992 meeting the current concerns of the CSBRC were the diminishing role of the public sector in maize breeding, the federal and state budgetary constraints causing decreased financial support of public programs, and the relative lack of public support for maize research as compared to other crops (such as small grains) despite the greater economic importance of maize to the nation. The CSBRC recognized the need to develop a Congressional lobby for corn research and find approaches for funding an extension of LAMP to enhance the best accessions. Supporting an enhancement proposal that would be used to lobby Congress for research money was seen as the best action that could ease the concerns noted above.

The CSBRC requested the public and private maize breeders who developed the initial enhancement proposals (Major Goodman; Randy Holley; Linda Pollak) plus Douglas Tiffany and Wilfredo Salhuana (now retired) of Pioneer Hi-Bred Int., Inc. to develop a combined proposal for a public/private collaborative effort to enhance the accessions identified as useful by LAMP. During 1993 the proposal was used to generate interest and solicit in-kind support from ASTA's member corn companies.

To develop the framework of public/private cooperation, correspondence was initiated by Douglas Tiffany with scientists known to have maize germplasm enhancement interest, asking them to pass the information along to others. From this correspondence, the Germplasm Enhancement (GE) network was compiled. This network has grown to include cooperators, administrators, politicians, and others, who receive notice of cooperator meetings, field days, updates to the GEM website, and other general information. At the GE network's first meeting held in association with the ASTA's Corn & Sorghum December 1993 meeting, a technical committee to oversee enhancement efforts was elected. The committee included Wilfredo Salhuana as chair, Linda Pollak as coordinator, and Randy Holley, David Harper from Holdens Foundation Seeds as coordinator of a lobbying effort, Douglas Tiffany of Pioneer Hi-Bred, Kevin Montgomery of Golden Harvest, and Blaine Johnson of the University of Nebraska (Salhuana et al, 1994). Jim Parks of Wyffels Hybrids was added later to represent ASTA.

Many have used mass selection to adapt tropical germplasm to temperate conditions (Hallauer and Sears, 1972; Longquist, 1974; Genter, 1976; Compton et al., 1979). But because the goal of this enhancement effort was for the products to be used in commercial programs as quickly as possible, we decided to adapt the germplasm by crossing to adapted inbred lines. During the winter of 1993-94, 46 LAMP accessions and tropical hybrids were crossed to the public inbred lines B73 and Mo17 of the Stiff Stalk (SS) and non-Stiff Stalk (nSS) heterotic patterns, respectively. Although these two inbred lines were no longer used commercially, they were considered by the technical committee to be the most representative of modern commercial inbred lines. The crosses were backcrossed to the same inbred line in 1994. These 50% and 25% breeding crosses were intended to be the initial breeding crosses from which enhanced  $S_3$  lines would be developed. During extensive discussions in developing a breeding protocol,

however, the technical committee realized that these enhanced lines would be much less productive than those that could be developed by using modern commercial inbred lines to make breeding crosses. Each of the private members requested and eventually received permission from their companies to use proprietary inbred lines to make breeding crosses, that would then belong to the cooperative project. This was an unprecedented use of proprietary inbred materials at this time, and demonstrated that the private cooperators were committed to making the project succeed.

In August of 1994, project and breeding protocols (using a modified pedigree breeding procedure) were sent to all ASTA companies with maize breeding programs asking for their willingness to participate in GEM. Participation as a private-sector collaborator in GEM involved signing an agreement and following the protocol. The protocol required them to:

- Contribute in-kind support for the breeding effort (winter and summer nursery rows, yield trial plots, and disease observation rows)
- Cross exotic accessions assigned by the coordinator to their proprietary inbred lines and return the crosses to the coordinator
- Not distribute proprietary x exotic germplasm outside the company.

The amount of donated in-kind support was determined by the company according to their investment in maize breeding. In-kind support by industry was considered important for lobbying the U.S. Congress, for providing the necessary number of testing environments, for ensuring that the enhanced materials will have commercial relevance, and for providing public programs with routine cooperation and guidance. In return, a participating company received early access to GEM materials. Proprietary concerns were addressed by the following points:

- A company's S<sub>2</sub> lines can be considered proprietary

- Pedigrees will be coded so that only the coordinator knows which company made a proprietary cross
- It is extremely difficult to extract an inbred line once it has been hybridized, especially with an accession
- Publicly-released material from company breeding efforts can be  $S_2$  synthetics, as opposed to  $S_3$  lines from public breeders.

Because the private-sector authors of the proposal, representing some of the largest U.S. companies, had already signed the agreement before it was sent to the ASTA membership, company concerns that access to their proprietary inbreds would endanger their competitive position were reduced.

The proposal was also sent to public-sector maize breeders giving them the opportunity to become collaborators, although they were not required to make breeding crosses (because the most elite U.S. inbreds are proprietary) or provide in-kind support (because they lacked the financial resources). Signing an agreement allowed them access to GEM materials, potential financial or in-kind support to perform specific research or conduct breeding programs, and signaled to the U.S. Congress and USDA-ARS their belief in the value of the project. The companies represented on the technical committee also considered that allowing public breeders access to the proprietary inbred line by exotic breeding crosses was a means by which modern commercial breeding material could be moved to the public sector. The technical committee knew that it was critical to obtain outside funding to help public cooperators participate.

In addition, the proposal was sent to the countries who participated in LAMP, inviting their cooperation. This was in recognition of the role the countries had played in identifying the germplasm used in GEM, and the largely Latin American source of the exotic germplasm.

However, even though the counties were interested in participating, it was impossible without outside funding. As of this date, funding for their participation has not been found even though their participation has been encouraged by the CSBRC.

Within a month and a half of the protocols and agreement being sent out, 17 seed companies and 14 public breeders had signed the agreement to become cooperators. The project has grown rapidly. In spite of rapid consolidation in the industry, in 2001 there were 39 cooperating companies. These companies included two starch processors, demonstrating the interest in value-added properties of the breeding materials. These companies included 11 popcorn companies who participated with several public cooperators in a popcorn sub-project of GEM. By 2001, 47 public scientists had signed the agreement cooperating with varying levels of participation. There were also two international cooperators who had also participated in LAMP, Brazil and Argentina.

A sub-committee from the CSBRC led by David Harper lobbied key legislators of the U.S. Congress for permanent base funding to ARS to support the public effort at ARS and university locations, which they estimated at requiring \$1,000,000 per year. Besides recognizing the value of the work, Congress was impressed by the \$1.5 million contribution Pioneer Hi-Bred International made to LAMP, and the in-kind support from companies for GEM that was estimated by the companies to be worth approximately \$450,000 per year in 1994. This amount underestimated the actual value of the contributions because it ignored the value of the proprietary germplasm, the overhead costs, and the advice and service of the private breeders. In 1995 \$500,000 of permanent yearly funding was appropriated by Congress to support coordination of the enhancement effort at the Corn-Belt ARS location in Ames IA, research on value-added traits in Ames, data management of the project at Ames, a satellite location at a

southern ARS location in Raleigh NC, and support of public cooperators at other ARS and university locations.

### **C. GEM's Objective**

The objective of GEM is to provide to the maize industry materials developed using germplasm enhancement of useful exotic germplasm, with the ultimate aim of improving and broadening the germplasm base of maize hybrids grown by American farmers. GEM is an ongoing project, but to initiate enhancement 51 elite tropical and temperate LAMP accessions were chosen, plus 7 commercial tropical hybrids provided by DeKalb Genetics. The enhancement protocol is for one of the private cooperating companies to cross an exotic material by a proprietary inbred line to make a 50% exotic breeding cross, then for another private cooperator to cross the 50% cross with their proprietary line of the same heterotic pattern to make a 25% exotic breeding cross. All 50% and 25% breeding crosses are evaluated for yield as testcrosses, and the best used to develop breeding lines by cooperators. Because proprietary germplasm is used to make breeding crosses, access to breeding materials is limited to GEM cooperators but the opportunity to become a cooperator is available to all. Data collected on GEM materials is freely available, and GEM enhanced lines and synthetics will be freely available through the U.S. North Central Regional Plant Introduction Station (NCRPIS) after their public release. Traits targeted for improvement are agronomic productivity, disease and insect resistance, and value-added characteristics.

## **III. GEM's ADMINISTRATION**

### **A. Organization**

The organizational structure of GEM was based on LAMP. All GEM cooperators function similarly to the LAMP Principal Investigators, by being responsible for the project's

execution. A cooperators' meeting is held once a year at the Corn & Sorghum ASTA meetings to discuss progress. Cooperators have the opportunity to view breeding materials at a field day held each year at Ames. Occasional field days are held by other cooperators. Public cooperators include both federal (USDA-ARS) and university faculty members, and include breeders, entomologists, plant pathologists, animal scientists, and food scientists. A technical steering group (TSG) meets three to four times a year to discuss policies, protocol, and results. The TSG is composed of seven members from industry representing large, regional, and foundation seed companies, and one public cooperator representing a university. USDA-ARS attendees were at first limited to two (GEM coordinator and North Carolina project leader) but have since increased to include others involved in the project. USDA-ARS attendees are ex-officio due to conflict of interest. Members from companies and universities serve a staggered three-year term. Wilfredo Salhuana and Linda Pollak have served as chair and coordinator, respectively, and Marty Carson of USDA-ARS has served as North Carolina project leader, since the beginning of the project.

The GEM coordinator in Ames is responsible for what is to be done, when, and at what cost. The coordinator manages seed curation, manages line evaluations and release, plans and organizes cooperative nurseries and yield tests using in-kind support, manages data analysis and management, and coordinates public cooperator research and finances. A world-wide web home page has been developed (<http://www.public.iastate.edu/~usda-gem/>) that makes it easy to learn about GEM, contact GEM cooperators, obtain data, and order seed. In addition, the Ames location conducts value-added trait research and develops enhanced lines for improved yield and value-added traits. The Raleigh project leader manages enhancement of the 50% tropical

breeding crosses for the southern USA and coordinates public cooperator projects in the southern USA.

### **B. Funding Mechanism**

GEM has received \$500,000 from the federal government each year since the U.S. Congress's appropriation in the 1995 budget. After overhead, the Ames location received approximately \$300,000 and Raleigh received approximately \$150,000 each year. Of this amount, approximately \$60,000 from Ames has gone each year to help support public cooperators (Table 1). A comparable amount has been used from the Raleigh budget to help support southern public cooperators conduct yield tests and to support the breeding effort of Major Goodman. In-kind support from private cooperators each year provides approximately 6500 summer nursery rows, 2000 winter nursery rows, and 2600 disease observation rows. Over 7000 rows of yield test plots throughout the eastern, southern, and Corn Belt sections of the USA provide a wide diversity of environments. Additional GEM-related nursery rows and yield test plots are grown in the Ames and Raleigh locations.

## **IV. BREEDING ACTIVITIES AND RESULTS**

GEM's standard breeding protocol developed by GEM's Technical Steering Group is presented in Table 2. The numbers of  $S_1$ 's and  $S_2$ 's in Table 2 that need to be selected and evaluated are goals, and due to the unadapted nature of many of the breeding crosses are sometimes difficult to obtain. For example, a few of the breeding crosses have produced lines with a high percentage of male-sterile tassels, or lines with poor synchronization between male and female flowering. The coordinator has a great deal of latitude to help cooperators modify the protocol to fit their special requirements.

The LAMP U.S. Principal Investigator, Linda Pollak, and Technical Advisor, Wilfredo Salhuana, selected 51 highly productive LAMP accessions from temperate and lowland tropical areas as starting materials for GEM. In addition, DeKalb Genetics donated seven tropical hybrids. In 1994 the coordinator assigned a minimum of four accessions or tropical hybrids to each private cooperator to cross to a proprietary inbred line in the 1994-95 winter nursery. The coordinator specified the heterotic pattern (SS or nSS) to use based on previous information from LAMP testcrosses (Salhuana and Sevilla, 1995; Salhuana et al, 1998; and unpublished data). If the heterotic pattern of the exotic material was unknown, the coordinator had crosses with both heterotic patterns made.

Private cooperators returned exotic x private inbred breeding crosses to the coordinator from winter nurseries in the form of balanced bulks for future breeding and for making 3-way crosses, and leftover bulks for other evaluations. Balanced samples of the breeding crosses were sent to two different companies for making 3-way SS or nSS breeding crosses in 1995 summer nurseries or day-neutral locations. Each private cooperator was assigned a minimum of eight crosses. New private cooperators were assigned four accessions to make breeding crosses.

In the 1995-96 winter nurseries, two companies (Golden Harvest and Pioneer) made nSS testcrosses with all available SS breeding crosses, and two companies (Cargill and Holdens) made SS testcrosses with nSS breeding crosses. Cooperative yield testing during 1996 involved 16 experiments each grown in more than six locations, evaluating 564 testcrossed 50% and 25% exotic breeding crosses. From these results, breeding crosses were selected for advancement to line development. Results for the best topcrosses of Stiff Stalk breeding crosses and non-Stiff Stalk breeding crosses are shown in Tables 3 and 4, respectively, expressed in percentage over the five check hybrids (LH195/LH212 and LH195/LH59 from Holdens Foundation Seeds, and

Pioneer Brand Hybrids 3489, 3525, and 3163). Some results are similar to the mean of the checks or better, thus we expect the testcrosses of the best lines developed from these breeding crosses to be superior to the check hybrids. Any line selected through this procedure will probably need agronomic improvement so they are best used as breeding lines in a commercial breeding program.

In Ames and North Carolina before the above data were available, line development was started in a few breeding crosses based on LAMP data (Salhuana and Sevilla, 1995). In 1997 the lines were grown as testcrosses in yield tests in the Corn Belt and Southeast. Approximately 130  $S_2$  or  $S_3$  lines from GEM breeding crosses were better than the average of commercial check hybrids in trials managed by Raleigh, and approximately 65  $S_2$  lines had yields similar to or greater than commercial check hybrid means in trials managed by Ames (GEM, 2000). Results for some of these lines are in Table 5.

After the initial seasons of developing and evaluating breeding crosses, GEM's seasonal breeding program has developed into a balance of the above elements (developing and evaluating new breeding crosses), ongoing line development at various stages with public and private cooperators, and maintenance activities such as regenerating breeding crosses and increasing lines. To date, we have developed approximately 500 breeding crosses, and instituted pedigree breeding for line development in many of these. Yield data is available by CD-ROM for 1997-1999 experiments (GEM, 2000), and after that on our website (<http://www.public.iastate.edu/~usda-gem/>). Results indicate that these lines have true yield potential. For example, in experiments grown by Pioneer Hi-Bred Int., Inc. in 1998 to test  $S_2$  lines from CUBA164 breeding crosses testcrossed with a Pioneer non-Stiff Stalk inbred line, a CUBA164 topcross was the second highest yielding entry behind Pioneer Brand Hybrid 3525, in

six replications. Seven other lines yielded more than Pioneer 3163, ranking five to eleven after Pioneer checks 3489 and 34G81. In another experiment with seven replications, eight testcrosses from a CUBA164 breeding cross yielded more than Pioneer Brand Hybrid 3163, ranking two to nine after Pioneer Brand Hybrid 33A14. Another experiment of four replications grown by DeKalb Genetics evaluated lines from a 50% exotic breeding cross with an accession from Argentina, AR01150, testcrossed to a DeKalb Stiff Stalk inbred line. Six AR0150 topcrosses were the highest yielding entries, beating the highest yielding check, Pioneer 3163. Four more AR01150 topcrosses beat the next highest yielding check, DK621 (Pollak and Salhuana, 2001). Similar results have occurred for experiments testing lines from other breeding crosses in later years.

Each year's yield tests coordinated from Ames are a mixture of  $S_2$  testcross evaluations from public cooperators (primarily from the coordinator's breeding program, Linda Pollak), from private cooperators, retests of previously selected lines, and research experiments. Those yield tests coordinated from Raleigh are primarily evaluations of lines from the project leader's (Marty Carson) and primary southern public cooperator's (Major Goodman) breeding programs. From each year's public cooperator experiments in Ames approximately 10  $S_2$  lines per experiment are selected for further testing and possible future public release, approximately 50-60 total each year. As soon as the lines have been increased to  $S_3$  bulks, they are listed on our website and available to GEM cooperators for crossing and evaluating with their own inbred testers, for disease or insect evaluations, for breeding, and for research. Similarly, public lines are available to cooperators from the Raleigh breeding program.

From GEM's inception it has been understood that participating companies can freely use in their breeding programs genetic materials obtained either through seed orders of breeding

crosses or S<sub>3</sub> lines for possible future release, or through protocol breeding assignments as long as mutually agreed upon materials are returned to the coordinator. Companies are encouraged but not required to share data from evaluating materials through seed orders, or data taken on protocol breeding materials that was not part of the protocol.

## **V. VALUE-ADDED TRAIT ANALYSES AND RESULTS**

### **A. The Need for Improving Value-Added Traits**

Although the Latin American Maize Project (LAMP) was the first coordinated international project for evaluating a major world crop (Salhuana et al., 1991; Pollak, 1993; Eberhart et al., 1995), only grain yield and agronomic data were collected (Salhuana and Sevilla, 1995; Salhuana et al., 1997). Studies indicate that significant variability for quality (Tello et al., 1965; Jellum, 1970; Zuber et al., 1975; White et al., 1990; Hameed et al., 1994; Campbell et al., 1995; Dunlap et al., 1995) and biomass for paper pulp production (Hammes and Pendleton, 1984) is present in maize genetic resources. Because much of the exotic germplasm has undergone selection for many indigenous uses (feed, foods, beverages, etc.) by various cultures, it seems likely that new grain quality characteristics will be found in exotic rather than the narrow-based germplasm now used. Limited variability for feed quality is found in present-day hybrids and thus in elite breeding materials, according to composition trait values collected in the Iowa Corn Yield Tests since 1988, and 7399 samples collected from 27 locations in North America from 1987 to 1993 (Ertl and Orman, 1994).

Breeding for high-yielding hybrids using limited genotypes with reduced variability results in a high degree of uniformity in grain type and nutritional content. Uniformity is valued by farmers but may not serve the needs of users and processors. Corn is the major feed for livestock and will continue to be important. The livestock industry is becoming increasingly

concentrated as production moves from small independent operators to large integrated corporations thus are beginning to reach the point where minor changes in raw materials can provide significant changes in overall costs (Wheat, 1992). As more foreign customers demand meat products to improve the diets of their people, an increase in grain output becomes vital. The USA exports as much as 20 percent of its corn crop with over one-half of this export grain fed to livestock (Nutrient Content and Feeding Value of Iowa Corn, 1991). Hill (1981) emphasized the need for improved corn quality traits if the United States is to maintain its present world market export share.

### **B. The Value-Added Trait Research Component of GEM**

During LAMP, the USA Principal Investigator had a laboratory with analytical capability to analyze oil quality of corn kernels, and had research colleagues with analytical capability to analyze seed composition, wet milling characteristics, and starch quality. Along with the agronomic LAMP evaluations, many LAMP and other accessions and resulting breeding materials developed from the accessions were analyzed for these value-added traits (Campbell et al., 1995; Dunlap et al., 1995; Hameed et al., 1994; Ng et al., 1997; Pollak and White, 1997; and White et al., 1990). These studies indicated that there was great potential for improving adapted corn for these traits by introgressing exotic materials. Therefore, including a value-added research component to GEM was a primary objective during GEM's organization (Pollak, 1997). Support for value-added research of GEM breeding materials, additional analytical equipment, and a laboratory manager was included in the lobbying effort from the beginning.

At present the value-added trait laboratory in Ames has capability to measure starch, oil, and protein composition, starch quality (thermal and viscosity) characteristics, amino acid composition, fatty acid composition, and other traits such as tocopherol, carotenoid, and vitamin

levels. All GEM breeding crosses and the selected S<sub>3</sub> lines are evaluated for grain composition, with those close to targeted values undergoing further starch, oil, or protein quality evaluations as appropriate. Those lines with unusual traits close to targeted values are then included in the value-added research and breeding component of GEM as appropriate. All released S<sub>3</sub> lines will be released with all data that has been collected on them in the laboratory, thus the lines can be targeted to their intended use in commercial breeding programs.

### **C. Grain Composition**

Corn kernels are composed of approximately 73% starch, 10% protein, and 5% oil, with the remainder made up of fiber, vitamins, and minerals (Eckhoff and Paulsen, 1996). Corn's contribution as a feed ingredient is primarily as energy provided by starch. Among feed grains, corn is one of the most concentrated sources of energy, containing more metabolized energy-or total digestible nutrients-because of its high starch-low fiber content (Watson, 1987). The major drawback of corn, however, is its low protein content. In addition, the protein is of low biological value, as it does not supply the essential amino acids either in adequate quantities or adequate proportions (Perry, 1988). Because of its chemical composition, corn protein itself is not sufficient for optimum animal growth. The total protein of corn is deficient in lysine and tryptophan for monogastric animal species and requires supplementation for adequate performance (Church and Nipper, 1984; Watson, 1977). One advantage of corn is that it has high levels of the S-containing essential amino acids, cystine and methionine (Watson, 1988).

To increase the energy content other foodstuff such as food oil is added to the feed. As nutrient requirements become more accurately defined through research, it is possible to formulate diets more precisely (Nutrient Requirements of Swine, 1988), thereby achieving more efficient animal production. Since lipid provides more energy than starch (9 cal/g vs. 4 cal/g), it

is beneficial to increase the lipid content to provide more energy. The average oil content of corn is 4.4% of whole corn kernels (dry basis) (Watson, 1987). Corn lines with oil content of 18% have been reported (Weber, 1987). This information is convincing evidence that it is possible to increase the oil content in corn along with other composition changes to improve the nutritional status of corn.

In the feed industry, supplementation is made by adding foodstuff rich in lysine and tryptophan amino acids, such as oilseed, fish and component meat meals. When oilseeds such as soybean meal are added to a feed mixture, the high content of lysine and tryptophan in soybean meal compensates for their deficiency in corn protein, while the high cystine and methionine content of corn protein compensates for their low value in soybean meal (Watson, 1988). Supplementation usually takes up to 28% of the entire feed mixture (Watson, 1977), among which soybean meal is about 15% (Perry, 1988). If protein content in corn could be increased from 10% to 15% with more balanced amino acid contents, then, the use of soybean meal would be eliminated when 15% of protein is required in a feed mixture. This small increase of protein in corn could have a substantial economic influence for farmers because soybean meal is more expensive than corn (\$180 vs. \$120/ton, Church and Nipper, 1984). Taking into account 120 million tons of corn and 16 million tons of oilseed meals used for feed each year (Watson, 1988), the economic benefit to farmers is obvious.

All livestock producers, large or small, must use supplementation to achieve optimum growth of animals. Supplementation adds extra costs of ingredients, transportation, storage, and feeding complexity. One way to minimize this problem is to improve the nutritional values of corn by breeding. The effectiveness of incorporating exotic germplasm into Corn Belt material to alter value-added traits is illustrated in Table 6. In the Corn Belt the typical values of protein,

oil, and starch are 10%, 5%, and 73%, respectively (Ertl and Orman, 1994). Each step in our enhancement protocol, from the Argentine accession AR16035, to the breeding cross, to the lines developed from the cross, show additional improvement. Without selection for composition, lines from this breeding cross already nearly meet, equal, or sometimes even exceed our target composition values of 16%, 7%, and 75% for protein, oil, and starch, respectively. This example clearly shows that the Corn Belt material was effectively enhanced by exotic germplasm.

We use near infrared reflectance spectroscopy (NIRS) to measure corn composition. NIRS is a rapid, non-destructive method of measuring the nutritional properties of grain. The instrument has a standard calibration based on scans from a wide variety of maize germplasm. The values are substantiated with wet chemistry analysis of constituents and used to evaluate unknown samples. Our samples are analyzed as whole grain for protein content, oil content, starch content, and moisture. Grain from GEM breeding crosses and selected lines for possible release are estimated as predicted by the calibration data. These early breeding materials with best compositional profiles are used in the value-added trait breeding and research program at Ames. The targeted values for grain quality are protein of 13% or higher; oil of 6% or higher; and starch of 75% or higher. We usually try to select and advance both high and low values for these traits for research use. In screening 139  $S_1$  lines from a special project designed to develop a few lines (approximately 10) from many breeding crosses for value-added trait research and breeding, we obtained ranges of 2.0-6.8 for oil (%dm), 8.7-15.8 for protein (%dm), and 65-73.5 for starch (%dm) (unpublished data). In screening selected  $S_3$  bulk lines from 1997 and 1998 yield tests coordinated from Ames we obtained values of 1.9-5.3 for oil (%dm), 9.4-15.1 for protein (%dm), and 64.7-73.1 for starch (%dm) (unpublished data). NIRS is a good method for

screening for breeding material and as a selection tool in a breeding program, as two hundred samples can be non-destructively analyzed each day.

#### **D. Starch Quality**

Corn starch is widely used in food and non-food industries. Examples of corn starch use range from providing the functionality or properties of anticaking, dusting, molding, viscosity, texture, film-forming, colloid protection, sweeteners, or encapsulation in the food industry to supplying alcohol, ethanol, binders in gypsum board, additives in paper making, or serving as an ingredient in the development of thermoplastics and polyurethanes (Orthofer, 1994; White, 1994). Corn starches are mixtures of linear and branched polysaccharides, which give very different physical properties. The starches composed of only one component have special properties that can be utilized in broader applications and lead to specialized uses for which regular starches are not appropriate (Whistler, 1984; White, 1994). One approach to improve starch functionality is to chemically modify the physical and chemical characteristics of native starch to provide unique functionality. Still another approach is to develop corn varieties that naturally produce starches requiring less or even no chemical modification for unique use. This approach is especially valuable to the food industry as an opportunity to develop “all natural” convenience foods. Naturally-modified starch in normal corn hybrids could be beneficial to farmers if the large yield reduction that occurs with mutant corn is avoided (Pollak and White, 1997). Oil and protein have commercial value as by-products from the production of corn starch in the food industry.

Starch represents nearly 70% of the dry weight of the mature corn kernel and is the most economically important component. Therefore, it is essential to determine the endosperm variation and starch quality of selected GEM materials. The process has two steps. The first step

is the extraction of the starch from the endosperm of single kernels using a modified mini wet milling procedure developed in our laboratory (Krieger et al, 1997). The second step evaluates the starch qualities and structures by measuring the starch gelling properties with the differential scanning calorimeter (DSC) to predict functionality in a food or industrial use (Stevens and Elton, 1971; White et al., 1990; Pollak and White, 1997). The DSC allows direct measurement of the energy required to gelatinize starch. The gelatinized samples are held for a week in a refrigerator and rescanned to determine the amount of retrogradation or recrystallization and hence the stability of the starch gel. Because we use single kernel analysis, we do not use this analysis on breeding lines unless they are fairly inbred,  $S_3$  minimum. Lines with unusual DSC scans are used to develop specialty corn lines with different starch properties than normal corn starch, and give breeders indications as to which breeding crosses might produce lines with different starch properties. To more fully understand the uniqueness of these lines, research involving additional laboratory analyses can be done. These analyses include Rapid Visco Analysis (RVA) to determine the rheological and pasting properties (important for food applications), scanning electron microscopy to observe the starch granules in the kernel, image analysis to ascertain granule size and shape, and gel strength. Additional research can also be done to determine the starch structural properties that cause the starch to have unusual functional properties. Research that examines how thermal properties, as measured by DSC, relate to structural and functional characteristics of the starch is summarized by White, 1994. Using the published research along with industry consultation, Table 7 of thermal properties and their implications was compiled by Dr. Pamela White, a public GEM cooperator from the Department of Food Science and Human Nutrition at Iowa State University. Typical DSC values for checks

used in our laboratory and some GEM lines with outstanding values (in bold) are shown in Table 8.

### **E. Oil Quality**

Corn oil is commercially produced from corn germ as a by-product of wet or dry milling. The value of corn oil results mainly from the recognition of the importance of its high content of unsaturated fatty acids and flavor stability (Leibovitz and Ruckenstein, 1983; Watson, 1988). Corn oil is used primarily as a premium cooking and frying oil (Sonntag, 1979) without hydrogenation. This superiority of corn oil to other vegetable oils became important when Mensink and Katan (1990) reported that *trans* fatty acids in a diet increased total and low-density lipoprotein-cholesterol and lowered the high-density lipoprotein-cholesterol levels compared to *cis* fatty acids in a diet. The effect of *trans* fatty acids may be similar to that of saturated fatty acids which are believed to be the most effective dietary factors in increasing plasma cholesterol (Dupont et al., 1991). The majority of *trans* fatty acids (80%) in the human diet comes from consumption of hydrogenated vegetable oils, with the average per capita intake ranging from 6.5 g/day to 12.0 g/day of *t*FA (Borenstein, 1991). Oil with increased saturated fatty acid content may increase the corn oil oxidative stability further, resulting in a specialty oil suitable for deep-fat frying in the food industry or for margarine production without hydrogenation. Naturally saturated corn oils also should have fewer processing costs and should result in more profit for the farmers and/or less cost for the consumers.

Oil quality is determined by fatty acid composition measured on individual kernels. Because this analysis is done on individual kernels, it is usually reserved for fairly inbred breeding materials, usually S<sub>3</sub> lines. Corn oil from individual kernels is extracted following a procedure modified from soybean (Hammond, 1991). Individual kernels are placed in an

aluminum crushing plate in a hydraulic press and crushed. Hexane is used to extract the oil (Hammond and Fehr, 1984). Sodium methoxide in methanol is used to transesterify the fatty acids to fatty acid methyl esters (FAMES) which are analyzed on a gas chromatograph. Standards of the FAMES are injected to determine their retention times. There are certain parameters of importance indicating oil quality, such as high saturated fatty acid content (palmitic plus stearic acids), low saturated fatty acid content, and oleic acid. Corn usually has fatty acid values of palmitic 11.0%, stearic 1.7%, oleic 25.8%, linoleic 59.8%, and linolenic acid 1.1% and total saturated fatty acids of 12.7% (Strecker et al., 1996). The targeted values for lines for our breeding program are lines with <6% for low total saturated oil, >17% for high total saturated oil, and >65% for oleic acid for high monounsaturated oil, with our eventual goals for developing specialty oils that may be used in an application listed in Table 9.

## **VI. PUBLIC COOPERATOR RESEARCH AND RESULTS**

Research conducted by public cooperators usually is related to their area of expertise and covers a wide range (Table 1). The following research areas are priorities set by the TSG.

- First and second generation European corn borer (ECB1 and ECB2, respectively) resistance
- Corn rootworm resistance
- Gray leaf spot resistance
- Stewart's wilt resistance
- Anthracnose stalk rot resistance
- Fusarium ear rot resistance
- Virus resistance

- Silage quality
- Grain quality

### **A. European Corn Borer Resistance**

The GEM-related research of Drs. Craig Abel and Richard Wilson, entomologists at NCRPIS in Ames (now USDA-ARS at Stoneville, MS and retired, respectively) focused on the European corn borer, which causes large losses in maize. It is important to find exotic sources of resistance and study ways to introgress it into elite material. Peruvian LAMP accessions previously found resistant to leaf feeding by ECB1 (Abel et al. 1995) were evaluated for resistance to ECB2, corn ear worm, southwestern sugarcane borer, and western corn root worm (Wilson et al. 1995). The LAMP accessions Lambayeque 42, Piura 208 and Libertad 3 were resistant to ECB2 and corn ear worm, whereas Lambayeque 29 was resistant to ECB2 and western corn root worm (Wilson et al., 1995). The multiple pest resistance showed that these accessions would be useful in pest management.

The Corn Belt germplasm sources of ECB resistance are based primarily on the chemical DIMBOA but the resistance of these Peruvian accessions is not based on DIMBOA (Abel and Wilson, 2000). Larval feeding tests indicated that water-soluble factors from the Peruvian accessions inhibited the growth, development time, and survival of the ECB larvae (Binder et al., 1999).

The accessions showing resistance were crossed to two public inbred lines (B94 and B97). Public lines were used instead of private lines because the initial crosses were made before 1994, when GEM received permission from private companies to use proprietary germplasm in crosses. Plants of the F<sub>1</sub> generation were infested with neonate ECB larvae and only the resistant plants were backcrossed to lines B94 or B97. The same procedure was utilized to make the

second backcross using only resistant plants. Seed of the second backcross was planted and plants were selfed. Descriptive statistics for the evaluation for ECB1 and ECB2 in the best plants for first and second backcrosses (BC1 and BC2, respectively) (Table 10) show that ECB resistance was maintained through introgression. ECB1 scores are qualitative and range from 1 to 9. Ratings of 1-3 are considered resistant, ratings 4-6 intermediate, and 7-9 susceptible. ECB2 values represent inches of stalk tunneling; resistant 0-6 inches of feeding, intermediate 6-12 inches, and susceptible more than 12 inches. The mean, minimum and maximum values for the plants in BC1 and BC2 show very good resistance for ECB1 and ECB2. The lines developed from the second backcross with B94 were topcrossed with a nSS inbred and the lines with a B97 background were topcrossed to a SS inbred. Yield trials of these topcrosses were grown in 1997 GEM in-kind yield tests to evaluate yield and other agronomic characteristics. We expected that the best yield of the topcrosses would not be superior to commercial hybrids because the resistant Peruvian accessions were not among those LAMP accessions selected for high yield, and public lines were used to make the breeding crosses. However, three topcrossed lines had mean yields over six Corn Belt locations equal to or exceeding the mean yields of the five check hybrids (Holden's Foundation Seeds LH195/LH212 and LH195/LH59, Pioneer Brand Hybrids 3489, 3525, and 3163), and still exhibited ECB resistance.

The lines resistant to ECB were used in a breeding program to develop germplasm lines for insect resistance. Resulting lines were resistant to leaf feeding and collar feeding by ECB but had low levels of DIMBOA (Abel et al., 2000a). Evaluation of 15 experimental lines from this program were evaluated for corn earworm, fall armyworm, southwestern corn borer, and sugarcane borer (Abel et al., 2000b). Lines were found that were resistant to leaf feeding by fall armyworm and leaf and stalk feeding by southwestern corn borer. A line resistant to corn

earworm showed maysin levels lower than those commonly found in earworm-resistant lines, indicating a new source of resistance. One of the ECB resistant lines became the first publicly-released line from GEM (Abel et al., 2001).

### **B. Characterizing LAMP accessions and their crosses for Wet-milling Efficiency**

The GEM-related research of Drs. Suvrat Singh and Lawrence Johnson, food scientists at Iowa State University (Dr. Singh now at Ruiz Foods) focused on wet milling efficiency and other value-added traits. Forty-nine LAMP accessions most of which were among the first group of accessions used in GEM, two commercial hybrids (Pioneer Brand Hybrids 3394 and 3489), and two inbreds (B73 and Mo17) were evaluated for their compositional, physical, and wet-milling properties (Singh et al., 2001a). The recovered starch was characterized to identify any unusual thermal, pasting, gelling, or retrogradation properties (Singh et al., 2001c). Heterosis of these traits was examined in 10 selected GEM accessions crossed with each of Mo17 and B73 inbreds (Singh et al., 2001b; unpublished data).

The accessions contained 3-6% less starch, 4-6% more protein, and up to 2-4% more fat than the commercial hybrids (Singh et al., 2001a). Higher protein and fat contents make GEM lines energy dense and good for animal feed. On average, absolute densities were greater (1.32 versus 1.29 g/cc). and 1000 kernel weights were less for GEM than for the commercial hybrids; test weights were similar. Absolute density is highly correlated with protein content and kernel hardness. U.S. dent corn in general is quite soft, while the accessions are much harder, making them less susceptible to breakage and more suitable for dry milling. Thus the exotic genes may contribute these traits to the GEM lines.

The wet-milling characteristics of the GEM accessions were not nearly as good as for the commercial hybrids (Singh et al., 2001a). Starch yields averaged only 54.3% for the

GEM accessions versus 64.8% for the commercial hybrids. Proteins content of starches recovered from GEM were much greater than for commercial hybrids. Gluten yields were much greater while gluten protein contents were much lower for the GEM accessions than for the commercial hybrids due to difficulty in separating starch from gluten. Occasionally, high fiber yields were also obtained for the accessions, indicating that the starch did not separate well from the fiber.

Thermal properties of starches recovered from GEM accessions had much wider variation than starch recovered from normal dent corn (Singh et al., 2001c). These differences were statistically significant but not of practical significance to the starch industry. However, these differences can be useful to corn breeders to expand genetic differences in starch which would then be of value to the starch industry. Starches isolated from GEM accessions had on average higher gelatinization temperatures, lower heats of gelatinization (enthalpy), and similar percentages of retrogradation. GEM accessions had on average greater temperatures at peak viscosity, greater peak viscosities, and greater viscosity break downs. The gel strengths were typically greater for the GEM starches than for starches from commercial hybrids.

Ten accessions were selected from the 49 original GEM lines and crossed with two dent corn inbreds, B73 and Mo17 (Singh et al., 2001b). When crossing with B73, protein contents and absolute densities were greater for the cross than either parent; all other compositional, physical, and wet-milling properties were similar to the mean of the parents. When crossed with Mo17, starch contents, absolute densities, starch yields, and starch recoveries were greater, and gluten yields were lower than either parent; all other compositional, physical, and wet-milling properties were similar to the mean of the parents.

Mo17 expressed poor wet-milling properties per se but produced superior crosses than did crosses with B73, which had better wet milling properties as an inbred per se. Because Mo17 belongs to the non-Stiff Stalk heterotic pattern, breeders utilizing GEM lines to improve wet milling characteristics may want to focus on using the lines from non-Stiff Stalk breeding crosses.

Similar results for starch properties were observed using both inbreds (unpublished data). Gelatinization peak temperature of starches from the crosses were similar to those of starch from the accessions. Enthalpies, peak height indices, peak viscosities, viscosity break downs, and percentages of retrogradation were greater for the crosses than for either parent. Gel strengths were similar to the mean of both parents.

### **C. Other Significant Public Cooperator Findings**

Some other significant findings by public cooperators include the following:

- Mary Carson, the Raleigh Project Leader, found many 50% tropical GEM breeding crosses as well as advanced S<sub>2</sub> and S<sub>3</sub> lines that had Gray Leaf Spot resistance equal to the most resistant commercial check hybrid.
- In a trial to estimate silage yield and nutritive value grown in 3 replications at two Wisconsin locations, a testcross of DXL212:N11a-3182-1 had the highest forage yield and milk/acre in the trial (9.35 tons/acre and 23,541 lbs milk/acre). Milk/acre was estimated based on MILK2000 equations ([www.wisc.edu/dysci](http://www.wisc.edu/dysci)) developed by the University of Wisconsin Agronomy and Dairy Science Departments. A testcross of CUBA117:S1520156 also had excellent nutritional characteristics mostly due to low neutral detergent fiber and high in vitro neutral detergent fiber digestibility. This work was done by the public cooperator James Coors at the University of Wisconsin. In previous trials he found two testcross with

both excellent yield and quality: a testcross with CUBA164:S15-64-10 and a testcross with CUBA164:S15-184-1. The former had the highest silage yield in that trial, which included check hybrid N4687, one of the highest yielding silage hybrids currently available in the north central region of the U.S. Furthermore, both GEM topcrosses had above average quality for all traits examined (low NDF, ADF, and high IVTD, IVNDFD, and protein). In particular, the CUBA164:S15-64-10 testcross had excellent digestibility on both a whole-plant and fiber basis.

- Mark Campbell, a public cooperator at Truman State University, is developing lines from GEM breeding crosses that have been converted with the recessive amylose-extender (ae) allele. His goal is to develop lines that have starch-amylose values at least 65% amylose. Numerous selections from GUAT209:S13 x (Oh43xH99ae) and CUBA110:N1711 x (Oh43xH99ae) have starch-amylose levels at or exceeding 70%.
- Wenwei Xu, a public cooperator at Texas A&M in Lubbock, evaluated breeding crosses for insect resistance. His results showed that BVIR103:S04, DKXL380:S08a, DKB830:S19, GUAT209:N19, CUBA117:S15, and CUBA164:S20 may be new sources of corn earworm resistance.
- Bruce Hibbard, a public cooperator with USDA-ARS at Columbia MO found the breeding cross AR16026:N1210 less damaged than the insecticide control in rootworm evaluations. In European corn borer stalk tunneling evaluations, 51 breeding crosses were less damaged than the resistant check, Mycogen 7250, and in leaf feeding evaluations, 12 breeding crosses were less damaged than the resistant checks Mycogen 7250 and Pioneer Brand Hybrid 3184. Although no significant differences between GEM breeding crosses were found in a Western Corn Rootworm evaluation, all but one

cross were nominally less damaged than the susceptible control, B37×H84, and twenty-two crosses were nominally less damaged than the resistant control, NGSDCRW1(C4)S2.

- Jerry Sell, a public cooperator at Iowa State University, evaluated experimental high-protein GEM lines in chicken feeding trials. Overall, the data show that the greater protein content of the experimental corn could prove advantageous economically for use in feeds of broiler chickens because of a decrease in the amount of the major protein source (soybean meal) needed in diets containing these corns. Additional research needs to be done with larger supplies of the experimental corns to obtain more definitive information about their feeding value.

## V. CONCLUSIONS

### A. Factors Responsible for GEM's Successful Public/Private Collaboration

GEM provides the social returns (agricultural diversity) to justify its public support, and the potential for private returns to justify private participation (Knudsen 1998). One factor in its success seems to be its federal leadership which provides the funding certainty that would be difficult to achieve with university leadership and grant funding. This is critical because the project is too large to manage without funding. Funding certainty by the USDA is assured by the continued private lobbying. The USDA-ARS has incentives to support GEM because its size and national focus are some of the same reasons that the USDA involves itself in intramural research. Federal leadership also ensures secrecy of proprietary information, which private leadership would be unable to guarantee. The GEM developers were a small group of people who all knew each other, trusted each other, and communicated well with each other, so companies trust the coordinator and GEM staff to keep the identity of the proprietary lines in the

breeding crosses a secret. GEM protocol is written to give private cooperators confidence that their intellectual and proprietary rights will be safeguarded.

Another factor in GEM's success is its collaboration between the public and private corn breeding sectors. Each sector of maize breeding research can contribute according to their strengths. The federal sector contributes the national focus and funding, the university sector the specialized research projects that benefit regions or states, and the private sector the elite proprietary germplasm, in-kind support, and commercial relevance. The private sector can expect proprietary returns from their investment but yet also treat their enhancement contributions as a social good deed.

Increased intellectual property rights may have had a dampening effect on formal and informal information exchange among plant breeders, but information exchange among scientists is critical to creative problem solving. GEM's organization structure allows for formal and informal information exchange and leads to creative problem solving, in particular through the TSG and germplasm network functions. In the past, maize germplasm evaluation and enhancement was done on an inefficient piecemeal basis, making it difficult to obtain positive results. Largely because of the lack of positive results, germplasm work was relegated to a low priority. There was little interaction among the few scientists working in the area, and almost no sources of outside funding. Now there is an organized network of scientists interested in maize germplasm, and many chances to exchange ideas.

In-kind support has been critical to GEM's success. Donation of in-kind support reinforces the value the company places on the project, which is useful for lobbying. It also enhances participation of the companies involved, because they have no incentive to having their resources used inefficiently. Because the companies are so intimately involved in the project,

they may have been more willing to contribute their proprietary germplasm. In-kind support greatly increases the amount of breeding effort devoted to the project, increases the rate of developing potentially useful lines, and increases the number of testing environments needed to identify good lines.

Finally, GEM is careful to make sure that only the best germplasm is used. Exotic germplasm has been identified through LAMP, through companies (tropical hybrids), or through breeding programs outside the USA (e.g. CIMMYT). Other exotics used in GEM have undergone screening to identify traits important to maize breeders such as ECB resistance and value-added traits. Corn Belt germplasm included in breeding crosses is the best available: companies' proprietary inbred lines.

### **B. Extending GEM's Concept**

When the GEM proposal was sent to potential U.S. collaborators in 1994, it also was sent to the LAMP principal investigators asking them to collaborate. The LAMP countries had a large stake in the enhanced materials, because it was due to their efforts that the initial germplasm used by GEM was identified. Although the LAMP countries were interested in participating, it was impossible because of the lack of funding for an international project.

Although LAMP has been completed, the principal investigators meet every two years to exchange ideas and maintain their international research network. At the LAMP meeting in 1996 Dr. Wilfredo Salhuana, Technical Director of LAMP, Chair of GEM's TSG, and Research Fellow at Pioneer Hi-Bred Int., Inc. (retired), presented a proposal for a new international collaborative project named the International Maize Germplasm Enhancement Project (IMGEP) that would build upon LAMP and GEM (Salhuana et al. 1997). GEM breeding crosses would bring participating countries of IMGEP genes for improved yield, agronomic characteristics, and

value-added traits. Crosses made with participating international countries' improved germplasm would benefit GEM. The exchange of germplasm and the joint effort in the selection would amplify the benefit to other sectors or regions of similar environmental conditions and permit selection of traits besides yield important to the country. Participation in IMGEP could benefit maize-growing countries of the Asia-Pacific region. In a similar manner, incorporating the elements that made the public/private collaboration of GEM successful, the concept could be extended to other crops.

GEM's concept has already been extended to the public and private popcorn breeders in the USA. The popcorn group operates an enhancement effort to utilize dent GEM breeding materials and exotic popcorn populations in cooperation with the dent corn GEM. The popcorn breeders and companies are GEM cooperators and meet with the other cooperators at field days and cooperator meetings. The Popcorn Project Leader, Ken Ziegler, serves as an ex officio member of the GEM TSG.

GEM cooperators in Canada and the northern U.S. have been discussing the initiation of a more focused effort in the north with earlier breeding crosses, having found that most of the current GEM breeding crosses are too late for their locations. Formation of new breeding crosses targeted to the northern effort has begun. Some of the starch quality cooperators have also discussed the concept of a more focused effort.

One example of the concept of LAMP extended to other crops is the Soybean Asian Variety Evaluation project, Project SAVE (Manjarrez-Sandoval et al., 1996). This project evaluates modern Asian soybean varieties for their potential as sources of new yield genes for U.S. soybean breeding. Collaborators include USDA-ARS and land-grant universities, with support provided by the United Soybean Board, a commodity group.

No single organization, nation, or region has the capacity to improve its agriculture to its optimum. There is a long history of international collaborations, germplasm exchanges, and mutual interdependence in agriculture. Our future depends on not only breeders but the general public and policymakers appreciating our mutual interdependence. As scientists, we must take the lead in developing collaborations that will safeguard our future.

### **C. GEM's Future**

GEM is a project designed to keep generating variability. The future of GEM is to continue contributing new germplasm with yield and other characteristics necessary for the demands of new markets. Since needs of farmers and industry change with time it is necessary to start looking into new genetic resources that may have the desired characteristics for their needs. Increasing productivity and quality, insect and disease resistance, tolerance to stress conditions, and additional traits that add value to the grain (starch, protein, oil, etc.) are characteristics that need to be improved in the future. We anticipate that new technology will facilitate transfer of useful genes from unadapted germplasm to elite material, and will help in searching the world's genetic resources for valuable traits.

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