Review

Breeding and disseminating quality protein maize (QPM) for Africa

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Quality protein maize (QPM) describes a range of maize cultivars with twice the content of limiting amino acids lysine and tryptophan compared with conventional maize, and has been developed to help reduce human malnutrition in areas where protein deficiency is prevalent and where maize is the major protein source in the diet, as in various parts of Sub-Saharan Africa (SSA). The International Maize and Wheat Improvement Center (CIMMYT), in collaboration with the International Institute of Tropical Agriculture (IITA) and the National Agricultural Research Systems (NARS) in 17 countries of SSA, has developed a broad range of QPM cultivars responding to the needs of different countries and agroecological zones. Commercial QPM seed is currently available in all collaborating countries and, based on average 2003-2005 seed production, approximately 200,000 hectares of land are being planted to QPM cultivars.

Key words: Quality protein maize, *opaque-2*, en-modifiers, aa-modifiers, genetics, plant breeding, dissemination, impact assessment, human nutrition.

INTRODUCTION

QPM breeding began with the objective of improving the nutritional value of maize grain protein. Normal maize protein, as a point of comparison, has a biological nutritional value of 40% of that of milk (Bressani, 1991) and therefore needs to be eaten with complementary protein sources such as legumes or animal products. Unfortunately, many millions of people worldwide are overly dependent on maize as a staple food through economic necessity. In Africa, maize supplies at least one fifth of total daily calories (Table 1) and accounts for 17 to 60% of the total daily protein supply of individuals in 12 countries as estimated by FAO food balance sheets. These values are average estimates per capita, and specific groups within these countries such as weaning children, sick children or adults, or all individuals during lean crop production cycles are even more dependent on maize as the major source of dietary protein and are therefore more susceptible to risk of protein or essential amino acid deficiencies. Research on QPM has been an ongoing study area for several decades but, while many papers were published in the early stages, in later years a real shortage of scientific documentation is being felt, particularly in the area of impact analysis at the community level. There has been minimal dialogue between maize breeders and nutritionists, economists, statisticians, and the extension and public health communities (Pinstrup-Andersen, 2000). Each of these sciences has developed new insights and methods and therefore when breeders work on improving nutritional quality it is critical to have open communication among these diverse fields. In this review we attempt to present the latest information on genetics and breeding methods of QPM, review various impact assessments and give an overview of actual dissemination of QPM with a focus on Africa. Based on recent experiences we conclude with the challenges ahead.

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Table 1. Importance of maize in the diet of individuals in selected African countries with respect to the percentage of calories and protein in the total diet.

Country	Maize as :	
	% Total Calories	% Total Protein
Lesotho	58%	55%
Zambia	57%	60%
Malawi	54%	55%
Zimbabwe	38%	46%
Kenya	36%	34%
Tanzania,	33%	33%
South Africa	33%	33%
Togo	25%	29%
Cape Verde	24%	26%
Swaziland	23%	24%
Mozambique	22%	31%
Ethiopia	21%	17%
European Union	1%	1%
United States	3%	2%
World	5%	5%

[a]Estimates calculated from FAO food balance sheets; FAOSTAT data, 2003.

EARLY QPM DEVELOPMENT

In the 1920s in a Connecticut USA maize field, a natural spontaneous mutation of maize with soft, opaque grains was discovered and delivered to the Connecticut Experiment Station (Vietmeyer 2000). This maize mutant was eventually named *opaque-2 (o2)* by a Connecticut researcher (Singleton 1939) and in the 1960's at Purdue University USA, the geneticist Dr. Oliver Nelson, (who began his career as a graduate student at the Connecticut Experiment Station (Crow et al. 2002), provided to Dr. Edwin Mertz seeds of *opaque-2* maize to be included in his group's systematic effort to identify maize accessions with improved protein quality (Paes and Bicudo 1994).

In 1961 the Purdue researchers discovered that maize homozygous for the recessive *o2* allele (with two copies of the mutation) had substantially higher lysine (+69%) in grain endosperm compared to normal maize (Mertz et al., 1964). It was further determined that this genotype also showed a corresponding increase in tryptophan content, and that the increased concentration of these two essential amino-acids (normally deficient in the maize grain endosperm) effectively doubles the biological value of maize protein (Bressani, 1991) with the considerably advantageous result that only half the amount *o2* maize (relative to normal maize), needs to be consumed to obtain the same biologically usable protein (FAO 1992).

Soon after the discovery of the nutritional benefits of the *o2* mutation, it began to be incorporated into many breeding programs worldwide, with a major emphasis on conversion of normal endosperm populations and inbred lines to *o2* versions through a direct backcross approach (Prasanna et al., 2001). However, enthusiasm over the direct use of the *o2* mutation in breeding programs soon subsided after the discovery of serious negative secondary (pleiotropic) effects of this mutation. The soft endosperm of *o2* genotypes initially caused up to a 25% yield loss due to the lower density of the opaque grains, as well as increased susceptibility to fungal ear rots and storage pests (Vasal, 2000). The soft endosperm texture also is not acceptable to many in the developing world who are accustomed to harder grain types. Such negative secondary effects severely limited practical use of the mutation in the field.

Fortunately, during the process of converting nor-mal maize populations to *o2* versions, partially hard endosperm (i.e. vitreous) or "modified" grains had been observed by many researchers including breeders at CIMMYT in Mexico. Separation of such grains when encountered began as early as 1969 by Dr. John Lonn-quist (Vasal, 2000). In addition, the first published report highlighting the importance of such grain modification in reducing the negative pleiotropic effects of the *o2* mutation was published in 1969 (Paez et al., 1969).

Selection for hard endosperm modification was rapidly incorporated into *o2* breeding schemes. Initial QPM breeding efforts at CIMMYT focused on conversion of a range of subtropical and tropical lowland adapted, normal endo-sperm populations to *o2* versions through a backcross-recurrent selection procedures, with a focus of accumulating the hard endosperm phenotype, maintaining protein quality and increasing yield and resistance to ear rot (Villegas et al., 1992). The improved populations were released for direct use in the field as open pollinated varieties (OPV's), or individual plants were self pollinated to form inbred lines used in hybrid formation. Similar programs with sustained breeding of QPM also continued at the University of KwaZulu-Natal (previously University of Natal), South Africa and the Crow's Hybrid Seed Company at Milford, Illinois USA (Prasanna et al., 2001). As a result many cultivars (both OPV's and hybrids) with improved protein quality were developed for temperate, tropical highland, and for subtropical and tropical lowland growing conditions. The resulting genotypes with elevated lysine and tryptophan content relative to normal maize but without the negative soft endosperm phenol-type were termed by CIMMYT as Quality Protein Maize (QPM). The term QPM now refers to maize homozygous for the *o2* allele, with increased lysine and tryptophan content but without the negative secondary effects of a soft endosperm. QPM looks and performs like normal maize (Figure 1) and can be reliably differentiated only through laboratory tests. It should be highlighted that QPM is the product of conventional breeding and no genetic engineering was used during its development.

QPM GENETICS AND BREEDING STRATEGIES

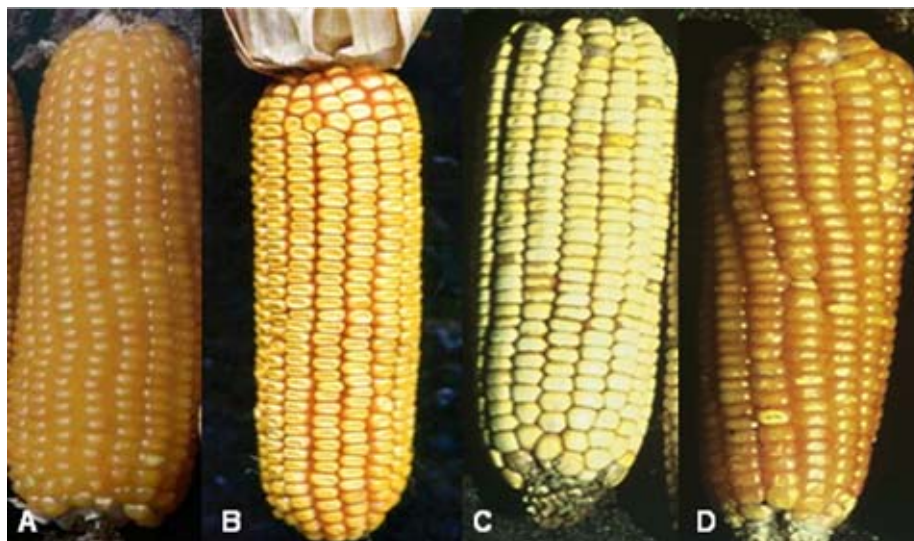The breeding of QPM involves the manipulation of three

Figure 1. Normal endosperm flint type maize (A), normal endosperm dent type maize (B), opaque-2 maize (C) and Quality Protein Maize (D).

distinct genetic systems. The recessive mutant allele of the *Opaque-2* gene is the first and central component. Characterization of this gene has identified it as encoding a transcription factor (a gene regulator) of zein synthesis (Schmidt et al., 1990). Zeins, and particularly alpha-zeins are the most abundant proteins in the grain endosperm (Gibbon et al., 2005) but are also characteristically poor in the amino acids lysine and tryptophan. The homozygous *o2* mutant causes a decrease of the production of these zeins resulting in a corresponding increase in non-zein proteins, which naturally contain higher levels of lysine and tryptophan (Gibbon et al., 2005). The *Opaque-2* transcription factor also controls the production of the enzyme involved in free lysine degradation, thus in grains with the *o2* mutation, a dramatic reduction in this enzyme leads to a corresponding increase in free lysine in the grain endosperm (Brochettobraga et al., 1992).

The second distinct genetic system managed within a QPM breeding program is comprised of the alleles of endosperm hardness modifier genes (termed here "en-modifiers") which convert the soft/opaque mutant endosperm to a hard/vitreous endosperm with little loss of protein quality. It has been shown that increased levels of gamma zein likely contribute to the recovery of a hard endosperm phenotype as the *o2*-modified (QPM) grains have approximately double the amount of gamma zein in the endosperm relative to the *o2*-only mutants (Wallace et al., 1990). These en-modifiers along with the *o2* mutant allele can be selected for using a rapid and low cost method of selection, whereby light is projected through the vitreous grains or blocked by the opaque grains respectively. Grain endosperm opaqueness is rated on a scale from 1 (=completely hard/vitreous) to 5 (= soft/opaque) (Figure 2). All grains with a score of 2-5 are homozygous for the *o2* allele, but only grains with

score 2-3 have sufficiently modified hard endosperm to be selected as QPM grains. Using this semi-quantitative measure, two genetic loci which affect the modification of the endosperm hardness in *o2o2* backgrounds have been mapped to the long arm of chromosome 7 (Lopes et al., 1995) and interestingly one endosperm modifier locus maps near a gamma zein gene *'gzr1'* (Lopes et al., 1995)(Maize Genetics/Genomics Database www.maizegdb.org). Genetic variance accounted for by these two major loci was not calculated in the study but it is likely that other en-modifier loci are also involved in the endosperm hardness modification.

The third genetic system critical to a QPM breeding program is comprised of a distinct set of amino acid modifier genes (termed here "aa-modifiers") which affect the relative levels of lysine and tryptophan content in the grain endosperm. The lysine levels in normal and QPM maize average 2.0% and 4.0% of total protein in whole grain flour respectively, but range across genetic backgrounds from 1.6 - 2.6% in normal maize and 2.7 - 4.5% in their *o2* converted counterparts (Moro et al., 1996) (Table 2). Lysine and tryptophan levels are highly correlated (Hernandez and Bates 1969) and as such an assay for either amino acid can be used for analyzing protein quality, although in practice the latter is most often chosen due to lower laboratory costs. Multiple genes have been identified in controlling amino acid content. At least three gene loci have been implicated in controlling the levels of a protein synthesis factor correlated with lysine levels and these have been mapped to locations on chromosomes 2, 4, and 7 (Wang et al., 2001; Wu et al., 2002). In the same genetic mapping studies, free amino acid content (including lysine) was measured using an alternative ninhydrin assay and nine significant loci were identified on chromosomes 1, 2, 3, 4, 5, 7, 8

Table 2. Lysine and tryptophan levels as a percentage of total protein in whole grain flour of normal and QPM, and FAO guideline requirements for children.

Parameter	Normal maize	QPM	Requirement for Preschool child (2–5 years)[c]
Lysine[a]	1.6-2.6% (avg 2.0%)	2.7-4.5% (avg 4.0%)	5.8%
Tryptophan[b]	0.2-0.5% (avg 0.4%)	0.5-1.1% (avg 0.8%)	1.1%

[a]Moro et al. (1996).
[b]CIMMYT tropical lowland sub program.
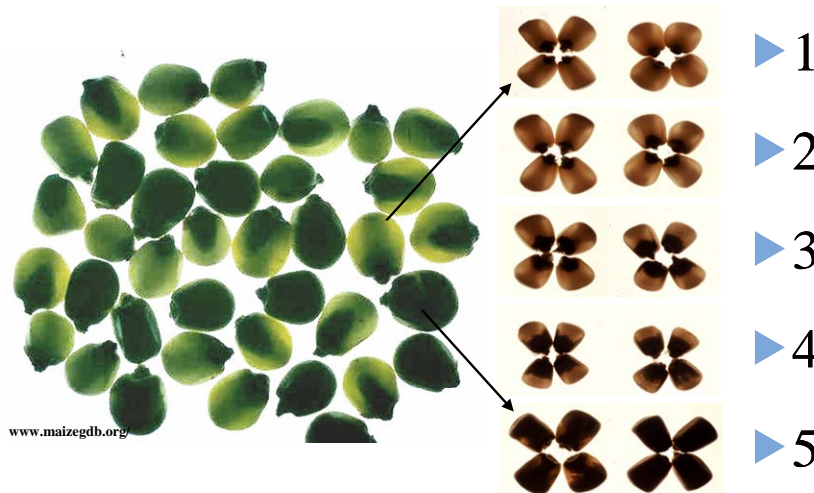[c]FAO 1985, Energy and protein requirements. FAO, Rome

Figure 2. Light table screen and classification of maize grains with variable endosperm hardness levels, ranging from vitreous/hard endosperm (1) to opaque/soft endosperm (5).
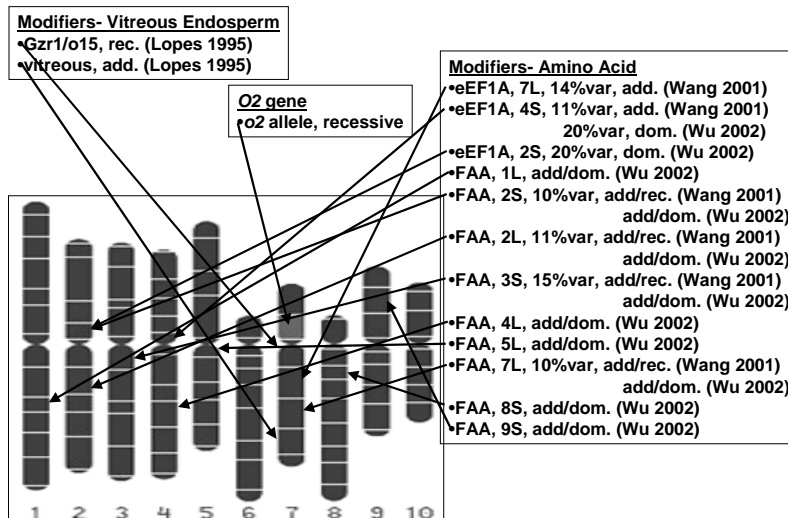

Figure 3. Various genes and quantitative trait loci which are components of the quality protein maize genotype.

*Abbreviations are defined as the following: '%var' = the percentage of the phenotypic variance accounted for by the genetic locus (if estimated), 'FAA'=free amino acid levels, 'eEF1A'=a transcription factor highly correlated with lysine and tryptophan levels in maize grains, 'add'=additive gene action, 'dom'=dominant gene action, 'rec'=recessive gene action.

Table 3. Yield, agronomic and grain protein quality of the normal and QPM versions of the hybrid CML264 X CML273 across six locations in 2002. Data taken from trial TSCWQ02-21.

Pedigree	Yield tons/ha	Endo Hard 1_5 [a]	Ear rot %	Days to Silk	Plant aspect	Ear aspect	Root Lodge %	Stalk Lodge %	Tryptophan % Total Protein
CML264 X CML273	5.27	2.2	3.8	56	2.8	2.9	6.6	2.4	**0.50** [c]
CML264Q [b] X CML273Q [b]	5.29	2.4	6.0	57	2.7	2.8	3.0	3.2	**0.91** [c]
Local Check #1	5.16	2.2	6.7	54	3.8	3.1	20.9	18.4	0.50 [c]
LSD 5%	0.65	0.5	4.5	0.8	0.5	0.3	10.4	8.9	

[a] 'Endo Hard 1_5'=Endosperm hardness scores based on visual inspection and on a scale of 1 to 5.
[b] Q indicates normal endosperm lines converted to QPM.
[c] Values assuming average of inbred lines and 10% protein on whole grain analysis.

and 9. As a result of these studies it has become appparent that the simple genetic nature of *opaque-2* maize has transformed into a classic polygenic trait in reference to QPM (Figure 3) and must be manipulated as such in breeding programs. If lysine or tryptophan levels are not continuously measured during the breeding process the additional gains in protein quality may be lost even though the *o2o2* genotype is maintained.

Current QPM breeding strategies at CIMMYT focus on pedigree breeding, whereby the best performing inbred lines, complementary in different traits, are crossed to establish new segregating families. New inbred lines are developed from these segregating families in the same process as from the broader based populations. Three types of crosses provide a choice of breeding strategies: QPM by QPM, QPM by Normal and QPM by Normal Backcross Conversion (of the normal genotype to QPM using at least three backcross generations). A modification of the second and third strategies is also often employed where by a single backcross generation to the normal endosperm parent is employed to increase the frequency of favorable yield and agronomic alleles, followed by selection within the resulting segregating family. Within each of these methods, successive inbreeding of the material is made in parallel with continual selection on the three QPM genetic systems, line *per se* performance and test-cross performance based on yield, agronomic characteristics and disease resistance. The primary end-products are inbred lines used in formation of QPM hybrids and QPM synthetic OPV's. The time required to develop an inbred line with the first two strategies is approximately nine cycles. However, if the breeding program has a sufficient pool of elite QPM lines, the first strategy is the simplest as the *o2* allele is fixed within the segregating family and selection is only required on en-modifiers and aa-modifiers and confusion of completely modified *o2o2* genotypes with normal genotypes can be avoided.

The breeding process of backcross conversion of a normal line to QPM is more complicated than the previous two methods. An F2 selfing generation is required between each backcross in order to fix the recessive *o2* allele prior to selection for en-modifiers and aa-modifiers.

As such, QPM conversion requires seven seasons to obtain the 3rd backcross at which on average 94% of the original (or recurrent) parent is recovered, whereas a typical direct backcrossing method for a dominant trait takes only four seasons to reach the 3rd backcross. However, even with the extended length of conversion of elite normal endosperm genotypes to QPM, the strategy is particularly useful when specific inbred lines are popular and widely used in hybrid combination, and therefore can greatly facilitate the adoption of QPM material in countries where specific normal endosperm hybrids are popular. While national registration procedures within individual countries would likely remain the same for such converted lines, this procedure would avoid the costly procedures of regional and international testcross yield trials of the breeding program as the converted line will essentially perform agronomically as the normal line (Table 3). In situations where adoption of a QPM version of a hybrid has difficulty competing in open markets with the original, the component QPM converted lines can be tested in different hybrid combinations with a goal of releasing new QPM hybrids with improved agronomic traits.

IMPACT ASSESSMENT OF QPM

Impact assessment of newly released maize cultivars are traditionally relatively straight forward using what are termed adoption studies. In these studies, the area planted in new cultivars is measured along with any increased yields on farmers' fields and the resulting increase in production is calculated. Economic surplus analysis (Masters et al., 1996) is then used to measure the benefits to consumers from the resulting reduced prices from surplus grain.

The economic analysis of nutritionally enhanced maize cultivars is particularly challenging however. Yield and other agronomic traits are usually assumed to be at least as good as conventional cultivars (so they would not impede adoption), and no particular yield improvement goals are set, making calculation of economic surplus inappropriate for new QPM cultivars. The major benefit of nutritionally enhanced maize such as QPM for human nu-

trition, however, is found in decreasing deficiency (of amino acids or micronutrients); As such, the improvement in the nutritional status and health of maize consumers, and the accurate measurement of that improvement is a much more difficult proposition.

In animal nutrition, QPM can provide a cheaper way of obtaining a balanced animal feed and that effect can easily be calculated in monetary terms. Its potential impact can be found by comparing the price of optimal feed ratios, typically calculated using Linear Programming, with QPM and with normal maize. Results for poultry and pig ratios in the US, using USDA feed requirement and average US prices for maize, soybean, sorghum and synthetic lysine and methionine, showed relatively modest cost reductions, higher for pigs (3.4% for meat pigs and 3.0% for sows) than for poultry (2.8% for broilers and 2.6% for layers) (López-Pereira 1993). A similar study in Kenya found a 5% cost reduction from substituting QPM for normal maize in broiler ratios (Nyanamba et al. 2003). In this study, the optimal ratios based on QPM and on regular maize, based on local ingredients and prices, were not only calculated but also formulated. Trials showed that broilers raised with either mixture had the same food intake, mortality, and growth. Extrapolating the 5% cost reduction over the broiler industry would translate into a gain of US$ 300,000 for Kenya. In situations common to small farmers, where a balanced nutrient animal feed is not used and maize is the primary or sole feed component, economic impact of substituting QPM for normal maize may be more significant, and studies in such scenarios still need to be conducted.

To measure the impact of QPM in human nutrition, multiple levels need to be considered. First, it needs to be established whether the improved protein quality of QPM results in increased protein utilization when consumed by children or adults. As will be shown below, this has been addressed primarily through a number of metabolic (nitrogen balance) studies. Next, QPM's impact on improving nutritional and health status needs to be demonstrated in target individuals and communities where malnutrition is prevalent, and where maize is a major component of the diet. The evidence so far is limited: this type of research is complex and while major insights can be gained from past research, especially concerning methodology, substantially more work is needed. Finally, to come to a comprehensive impact assessment, information needs to be gathered on the specific areas, and the number of people, that suffer from protein malnutrition and where maize constitutes the major protein source. In this field, little progress has been made to date.

Studies on the nutritional benefits of QPM and o2 maize began soon after the identification of the improved quality protein trait conferred by the o2 allele. The first batch studied children in clinical settings, with each trial only including a limited number of children. The protein quality of o2 maize and later QPM was evaluated using the nitrogen balance technique in a number of studies, such as those conducted from the 1960s through the 1990s by researchers in Guatemala (Bressani et al., 1969; Luna-Jaspe et al., 1971), Colombia (Pradilla et al., 1973), and Peru (Graham et al. 1980; Graham et al. 1989). In these studies young children recovering from malnutrition were fed o2 maize or QPM based diets as their only source of protein, and the results clearly showed the nutritional superiority of o2 maize and QPM over regular maize with respect to indicators such as apparent nitrogen retention and biological value. Similar studies in the United States evaluated the protein quality of o2 maize in adults and came to similar results (Kies and Fox 1972). These studies concluded that nitrogen balance and retention were higher with o2 maize and QPM, especially at lower levels of total protein intake. Moreover, unlike normal maize, young children can consume o2 maize in amounts needed for the positive nitrogen balance that is required for growth (Bressani et al., 1969). Recovering malnourished children fed QPM further showed the same growth as those fed modified cow milk formula (Graham et al., 1990). The studies described above in clinical settings show QPM's potential in a highly controlled environment; however, in the end it is necessary to demonstrate a positive impact on growth and health of target populations living in non-controlled environments and using maize consumed in typical diets.

Community level studies involving children consuming QPM versus normal maize in typical diets under normal conditions have been conducted in at least six countries to date, including India, Guatemala and Brazil, and more recently in Ghana, Mexico, and Ethiopia. The first of these, a six-month feeding trial conducted in 1975-1976 by the Indian Agricultural Research Institute (IARI), involved 134 children aged 18-30 months (Singh 1977). Various measures of growth suggested some positive benefit of o2 to normal maize, but statistical analyses were not conducted to establish significant differences between these two groups.

In 1976-1977, a study was carried out on nine Guatemalan coffee plantations to evaluate the impact of switching from normal maize to QPM production (Valverde et al., 1983, reviewed in Bressani, 1991). This study also cited benefits to child growth; however, it was published only as a technical report by the Instituto de Nutrición de Centro América y Panamá (INCAP), with evidence suggesting that confounding factors and interventions left the results difficult to interpret (Lauderdale 2000). A series of studies conducted in Brazil in the 1990s had similar problems (Paes and Bicudo, 1994). These studies were either discontinued or the data were never analyzed.

A more recent study conducted as a series of four community trials in the Ejura-Sekyedumase District of the Ashanti Region in Ghana was used to evaluate the effect of feeding infants a traditional maize porridge made from

either QPM or normal maize (Akuamoa-Boateng, 2002). The first two trials suffered methodological problems and were inconclusive. The third trial followed 422 children, aged 4 and 9 months, over a period of 12 months. The growth (weights and heights) and morbidity of these children were periodically monitored. The results indica- ted that children in the QPM group had significantly fewer sick days and less stunting, compared to children in the normal maize group. The fourth trial, conducted in May- December 2001 in the same district, evaluated the effects of both QPM and barley malt in the preparation of infant food, with the latter being added to increase the energy density of the food. There were four treatments (each with or without malt and with or without QPM), in which the growth of 600 children, aged 4-6 months, was monitored. The results showed significantly higher weight and height gains with the use of barley malt, regardless of whether QPM or normal maize was used to make the infant food. With the use of barley malt, weight gains were also significantly higher in the QPM group than in the normal maize group. These studies were published only as a report by the Ghana Health Service - Ashanti.

A study conducted in 2001 - 2002 in Oaxaca, Mexico, evaluated the effect of QPM versus normal maize con- sumption by malnourished children in four communities in the Mazateca and Mixe regions (Morales, 2002). Weights of 67 children under the age of 5 were monitored during the study, and differences were found between the two treatments in the proportion of children that recovered from malnutrition during the study period. Specifically, there was a change in physical development, as asses- sed by weight for age, in the QPM group but not the normal maize group. This study has thus far appeared only as a dissertation from the Colegio de Postgraduados in the state of Mexico, Mexico.

The most recent community study was conducted in April 2002 - October 2003 in the Eastern Wollega Zone of Ethiopia by the Ethiopian Health and Nutrition Research Institute (EHNRI) (unpublished). The study area was divided into four regions, with two allocated to QPM and two to normal maize. Growth was monitored in 160 young children of maize-producing families. Malaria struck the QPM house-holds disproportionately before the end of the study, negatively impacting the subjects' growth. The results therefore appear inconclusive and are as yet unpublished.

It can be concluded that the implementation of commu- nity level studies is very difficult. The studies reviewed here demonstrated problems in experimental design, data analysis, and sufficient sample size required for adequate power, especially when accounting for confoun- ding factors. This limits the conclusions that can be drawn about the effect of QPM (internal validity) or the generalizability of those conclusions (external validity) (Victora et al., 2004). It is recommended that future experimental research pays sufficient attention to the identification of potential beneficiaries, including the use

of secondary data and consumption studies. If the results show likely impact, they can be followed by community level experiments, where QPM is substituted for regular maize. These studies need to be carefully designed, with consideration of statistical issues arising in previous studies.

QPM DISSEMINATION IN AFRICA

A major challenge with QPM is the dissemination of the material into the farmer's field. Unfortunately, in the early 1990's the CIMMYT QPM breeding program was discon- tinued and as such the critical step of promoting this improved material was also severely limited. Since the late 1990's however, the Nippon Foundation of Japan and then later the Canadian International Development Agency (CIDA) have funded the continued improvement and promotion of QPM in several developing countries (Cordova, 2000).

In Sub-Saharan Africa, commercial QPM seed is currently available in 17 countries (Table 4) and based on average 2003 - 2005 seed production, approximately 200,000 hectares of land are being planted to QPM culti- vars. Breeding efforts have led to the release of one or more OPV's and/or hybrids in these countries although the total number of different materials is more limited since many releases share the same pedigree.

The country of Ghana has a long history of breeding for improved maize cultivars (Morris et al., 1999) and it is the dominant country for QPM production in Africa with approximately 70,000 hectares planted (Table 4). The vast majority of QPM seed produced is 'Obatanpa' (or improved versions there of) which was developed in collaboration with IITA from CIMMYT-developed lowland tropical population 63 converted to Maize Streak Virus (MSV) resistance and identified as 'Across 8363SR'. 'Obatanpa' was released in Ghana in 1992 and has since been released officially or is grown in 15 other African nations (Table 4) promoted largely by Sasakawa Global, 2000. Prompted by the success of 'Obatanpa' there was a renewed interest in development and dissemination of QPM in sub-Saharan Africa, supported by three comple- mentary projects funded by the Nippon Foundation (support for QPM germplasm development, dissemina- tion, and training) (CIMMYT, 2005), the Canadian Inter- national Development Agency (support for QPM develop- ment and QPM dissemination activities in Eastern Africa including socioeconomic and animal and human nutrition studies), and the Rockefeller Foundation (support for Eastern and Southern African scientists to initiate conver- sion of 19 widely-grown elite maize OPV's and hybrids to QPM).

Activities are led in West Africa by IITA and in Eastern and Southern Africa by CIMMYT, in collaboration with theAfrica Maize Network), ECAMAW (East and Central Africa Maize Network) and the SADC (Southern African

Figure 4. Growth rate advantage of pigs raised solely on quality protein maize (larger animals) versus sibling pigs raised solely on conventional maize (smaller animals). Separate feeding studies conducted in Guatemala, El Salvador, Colombia and Ghana in years indicated.

Table 4. QPM cultivars released in Sub-Saharan Africa.

Country	Hectares[a]	Cultivar	Type	Pedigree or Background	Traits[d]	Seed Production (tons)		
						2003	2004	2005
Benin[b]	4,325	Houlin-mi or Faaba	OPV	Across 8363SR	W, EI, F	73	100	
Burkina Faso[b]	20,600	Espoir	OPV		Y	513	311	
Cameroon[c]	305	Obatanpa	OPV	Across 8363SR	W, EI, F		6.1	
Cote d'Ivoire[c]	565						11.3	
		Obatanpa	OPV	Across 8363SR	W, EI, F			
		DMR ESR W QPM	OPV					
		EV99 QPM	OPV					
Ethiopia[d]	7,283	BHQP542 'Gabissa'	3WC	CML144/159//176	W, I, F	166	186	85
Ghana[b]	71,250					1,350	1,500	
		Obatanpa	OPV	Across 8363SR				
		Mamaba						
		GH-132-28	hybrid	P62, P63				
Guinea[b]	3,875					30	125	
		Obatanpa	OPV	Across 8363SR	W, EI, F			
		CMS 475						
		K9101						
		CMS 473						
		BR 473						
Kenya[d]	12					0	0.1	0.6
		WSQ104	OPV	Pool 15?	W,EE, F			
		KH500Q	3WC	CML 144/159//181	W, EI, F			
		KH631Q	3WC	CML 144/159//182	W, EI, F			

Table 4. Contd.

Malawi [b]	1,125	Obatanpa	OPV	Across 8363SR	W, EI, F	8	37	
Mali [b]	9,000	Obatanpa	OPV	Across 8363SR	W, EI, F	160	200	
Mozambique [b]	11,250	Susuma	OPV	Across 8363SR	W, EI, F	300	150	
Nigeria [b]	4,500					80	100	
		Obatanpa	OPV	Across 8363SR	W, EI, F			
		EV 99 QPM	OPV					
Senegal [b]	500					0	20	
		Obatanpa	OPV	Across 8363SR	W, EI, F			
		EV 99 QPM	OPV					
		DMR ESR W QPM	OPV					
		Susuma	OPV	Across 8363SR	W, EI, F			
South Africa [b]	12,500					250	250	
		Obatampa	OPV	Across 8363SR	W, EI, F			
		QS-7705	hybrid					
Tanzania [d]	4,300	Lishe-K1	OPV	Across 8363SR	W, E, F	50	83	125
		Lishe-H1	3WC	CML 144/159//176	W, EI, F			
		Lishe-H2	TopC	Obatampa//CML144/CML159	W, EI, F			
Togo [b]	750					10	20	
		Obatanpa	OPV	Across 8363SR	W, EI, F			
		EV 99 QPM						
Uganda [d]	46,717	Longe-5 'Nalongo'	OPV	Across 8363SR	W,EI, F	770	611	1,422
Zimbabwe		ZS261Q (CZH01021)	DC	CZL01006/CML176//CZL01005/CML181	W, F			
Total Africa	**198,857**							

[a] Hectarage based on average commercial seed production 2003-2005 at a conversion of 50 hectares/ton of seed.
[b] Source: Sasakawa Africa Association Annual Report 2003-2004; www.saa-tokyo.org/english/lastestinfo/index.html.
[c] Source: The Development And Promotion Of Quality Protein Maize In Sub-Saharan Africa, CIMMYT Progress Report to Nippon Foundation 2005.
[d] Source: Alpha Diallo personal communication.
OPV = open pollinated variety; TopC = topcross non-conventional hybrid; 3WC = three-way cross hybrid; DC = double-cross hybrid; hybrid = undefined hybrid; W = white grain; Y = yellow grain; F = flint; D = dent; S = semi-dent; EE = extra early; E = early; I = intermediate; L = late.

Development Community) maize breeding network (coordinated by the Southern Africa Drought and Low Soil Fertility Project, SADLF). In each sub-region, activities are highly integrated and coordinated enabling-joint development, exchange and broad testing of promising materials for all agro-ecological niches. QPM development in West and Central Africa currently is centered on an IITA initiated QPM breeding program started in 2002 - 2003 in collaboration with all member countries of WECAMAN (CIMMYT, 2005). The program involves optimizing the research strength of strong National Agricultural Research Systems (NARS) (lead Centers) by assigning them specific research problems. Lead Centers share germplasm and other technologies with the technology adapting NARS (weaker NARS). The program involves conversion of elite late, intermediate, early and extra-early maturing populations and OPV's and late maturing inbred lines (including *Striga* sp. tolerant germplasm) to QPM as well as QPM hybrid development. In addition, Ghana is converting the popular 'Obatanpa' to a yellow grain version and is converting

the high yielding normal endosperm yellow variety 'Sotu-baka' to QPM. Nigeria is converting 8 locally adapted inbred lines as well as the 'Acr Sakatifu' population to QPM.

The QPM breeding program for East Africa led by CIMMYT, in collaboration with NARS and small seed companies currently uses two broad approaches:

(i) Testing inbred lines, (both early generation and elite lines such as CMLs), hybrids, and OPV's developed primarily from CIMMYT-Mexico headquarters, as well as other breeding programs in, Ghana and South Africa, to identify the most adapted cultivars for direct release or use as breeding materials.

(ii) Converting existing popular adapted cultivars to QPM.

Considerable effort has also been dedicated to the formation of more streak resistant cultivars. Experience has shown that adequate resistance to MSV can often be achieved in hybrids by including one susceptible and one

resistant parent. For this reason, new QPM lines emerging from research at CIMMYT-Mexico which may be MSV susceptible can still be immediately useful in Africa when used in hybrid combinations with established MSV resistant QPM lines. CIMMYT work in Kenya aims to:

(i) Convert recently developed stress tolerant extra-early populations to QPM

(ii) Improve streak resistance in QPM populations from CIMMYT-Mexico.

(iii) Develop stress tolerant QPM lines using pedigree breeding in collaboration with NARS partners.

(iv) Introgress the Imidazolinone Resistant (IR) gene to QPM cultivars for *Striga* prone ecologies.

Currently more than 20 early QPM OPV's have been developed and distributed to collaborators through ECAMAW regional testing, along with numerous early and advanced generation inbred lines in test cross IR-QPM OPV's of tropical lowland adaptation are being made available to collaborators for evaluation in the *Striga* prone ecologies in sub-Saharan Africa. In Ethiopia, CIMMYT QPM cultivar development, carried out in collaboration with EIAR (Ethiopian Institute of Agricultural Research), focuses mainly on the highland zone, which has lagged behind other maize mega-ecologies. Currently under-going conversions to QPM are:

(i) Normal maize inbred lines with tolerance to *E. turcicum*/MSV/Gray Leaf Spot, (ii) Elite OPV's including four highland synthetics, (iii) Parental lines of released hybrids, including those of BH660, the most popular maize hybrid in Ethiopia, constituting nearly 95% of the nearly 10,000 t of hybrid seed produced annually in the country, as well as BH670 and BH540, (iv) For the non-highland areas, the popular OPV's 'Kuleni', 'Awassa-511', and 'Melkassa-1' are being converted to QPM and a rust and leaf blight tolerant 'Susuma' (a selection of 'Obatanpa') version is being developed.

Southern Africa QPM activities are largely coordinated by CIMMYT-Zimbabwe. Major breeding approaches are similar to the two outlined for East Africa with the specific aims of:

(i) Improve streak resistance in the best Mexican QPM materials, (ii) Develop new QPM germplasm by partially converting the best Southern African materials to QPM.

So far, about 1300 new MSV-resistant lines, based on the best Mexico QPM lines, and nearly 2000 experimental maize lines, developed from crosses between elite African germplasm (from CIMMYT and IITA) and Mexican QPM lines, have been selected for MSV resistance and advanced to further evaluation. Some exciting results include two MSV-resistant versions of CML144/CML159//CML176, a QPM hybrid released in several countries, including Ethiopia and Tanzania. As noted earlier, a sustained breeding effort of QPM has continued at the University KwaZulu-Natal, South Africa and additional QPM projects in South QPM hybrids in the Natal area, have released about 20 QPM hybrids and they have had good success using yellow QPM germplasm from Brazil and white QPM germplasm from Ghana.

The long-term goals of all breeding programs in the sub-regions are focused on broadening the genetic base of adapted QPM germplasm to suit their particular biotic and abiotic constraints. While Ghana and Uganda are the dominant countries for QPM production, several other Sub-Saharan countries are expected to increase their production in the next few years as the availability of competitive QPM OPV's and hybrids improves.

Conclusions

Currently several challenges confront QPM research despite the potential promise of its benefits in the field. While initially the greatest test was to close the yield gap between *o2* genotypes and normal endosperm counterparts, much of this work (along with significantly reducing ear rots and insect damage) was successfully accomplished in the 1970's and 1980s by the work of. Surinder K. Vasal and Evangelina Villegas (Vasal, 2000), who, in recognition of their accomplishments, received the World Food Prize in the year 2000. However, in some genetic backgrounds a yield difference of up to 5% can still be found and will need to be closed with focused breeding efforts.

While yield and yield stability of QPM germplasm is equivalent to normal endosperm counterparts and protein quality has shown considerable stability over diverse environments (National Research Council, 1988; Pixley and Bjarnason, 2002), it has been noted that some QPM genotypes under severe drought stress can significantly increase the frequency of soft or poorly modified grains relative to the same genotypes under optimal moisture growing conditions (Ngaboyisonga et al., 2006). Therefore particular attention to endosperm modification under drought conditions will need to be made in future breeding efforts.

The recessive character of the *o2* allele raises an additional challenge to the breeding strategy of backcross conversion as discussed above. Forward molecular marker selection for the *o2* allele has been suggested (Dreher, 2003) and implemented in attempt to achieve a faster conversion (Babu et al., 2005), and would appear to be an appropriate use of marker assisted selection (MAS). However, without concurrent selection on aa-modifiers, protein quality can drop considerably even within *o2o2* backgrounds. In the Babu et al. (2005) study, tryptophan content as a percentage of total protein decreased from

1.05% in the QPM donor line to 0.78 - 0.85% in the BC_2F_2 families. The reduction in protein quality when not selecting for aa-modifiers has also been observed within the CIMMYT QPM breeding program, and is not surprising considering the wide variation of lysine levels in *o2o2* genotypes of different genetic backgrounds (Moro et al., 1996). In order to bring to bear the full effectiveness of QPM genotype MAS (through reduced breeding time, selection out of main environment etc), a suite of effective markers linked to modifying loci of both endosperm hardness and amino acid levels needs to be identified. In addition, it is probable that additional traits will need to be coupled with selection for QPM to be cost competitive with traditional methods.

Currently, an unknown seed can be planted in the field, self pollinated and genotyped for seed traits (such as QPM) at a cost of $0.24/plant (assuming $6 and 25 plants per row). Current costs for marker genotyping are considerably higher at $0.50-2.50/plant USD. If the goal of multiple trait selection can be met then total MAS costs will be much more inline with traditional screening methods.

Accurate, rapid and cost effective measurement of grain amino acid content is a critical component of QPM breeding. While protein quality assessments via a ninhydrin (Zarkadas et al. 2000) and enzyme linked immunosorbent assay (Moro et al. 1995) have been used, the reliability of the assays can be low. CIMMYT has extensively used the colorimetric method of Hernandez and Bates (Hernandez and Bates 1969) but the reliability of this procedure is unduly dependent on the type of glacial acetic acid used in the process, which can vary greatly from one source to another. The colorimetric procedure therefore has limited use in countries with unreliable sources of this reagent. Research in substitution of glyoxylic acid for acetic acid in tryptophan quantification is ongoing at CIMMYT and any future assay that can lower the sample cost and reduce measurement error will be immediately useful in QPM breeding programs.

The recessive character of the *o2* allele raises an additional challenge to field implementation of QPM. If QPM is pollinated by pollen from normal maize, the resulting grain will lose the higher protein quality. Several years of field tests have shown that contamination of QPM open pollinated varieties is less than originally feared (Cordova, 2000). However, to ensure that the higher nutritional benefits of QPM are maintained, additional training of collaborators in partner countries with a focus on seed production techniques to maintain variety and line purity must also accompany QPM dissemination.

A future strategy to mitigate the contamination effects on the recessive character of the *o2* trait may lie with a transgenic approach. In separate evaluations, RNA interference (RNAi) was employed in gene silencing studies to reduce 22-kDa (Segal et al., 2003) and 19-kDa alpha-zeins (Huang et al., 2004; Huang et al., 2005) with anti-

sense transformation constructs. In these studies, lysine content was increased in the transgenic lines by 15 – 20%, although well below the 100% increase that is often observed in o2 genotypes. In a more recent however, an improved construct using double-strand RNA (dsRNA) was employed to increase the efficiency of gene suppression of both 19-kD and 22-kD alpha-zein gene families (Huang et al., 2006). As a percentage of total protein, up to 5.62% of lysine and 1.22% of tryptophan were achieved in transgenic lines compared with 2.83% of lysine and 0.69% of tryptophan in wild-type, which is above what is typically achieved with QPM lines and near or above FAO guideline requirements of lysine and tryptophan content (5.8 and 1.1% respectively) for children (Table 2). In these studies, the reduction of alpha-zein protein synthesis induced an opaque endosperm phenotype, which (as with the opaque phenotype caused by the *o2* allele) would need to be modified to a hard endosperm phenotype utilizing modifying loci. The dominant nature of the gene silencing transgene would be advantageous over the recessive *o2* allele when deployed in farmers' fields, as the high lysine/tryptophan levels would be essentially maintained even under relatively high amounts of pollen contamination from conventional maize. However, with this transgenic approach to improving protein quality, constraints on deployment of these materials in some countries, due to legislative barriers and lack of consumer acceptance would have to be confronted.

The invisible nature of the quality protein trait could also affect QPM adoption. If a QPM counterpart is equally acceptable and performs agronomically equal to existing normal varieties, farmers may continue to cultivars to which they are accustomed, rather than adopting the new ones, unless they perceive an advantage to adopting maize with the quality protein trait. Such an effect is a possible cause of why some QPM hybrids developed for Central America, why yielding similar to older normal endosperm hybrids, could not displace the latter (Hugo Cordova, personal communication). Hence, to facilitate adoption of QPM it would be preferable if it is agronomically better than the non-QPM to compete in open markets, or specific purchase agreements must be established between farmers and grain buyers (such as pig or chicken producers) in order for farmers to receive a premium price for QPM.

Adopters of QPM must have adequate knowledge of maintaining the quality protein trait in their fields, especially if seed will be harvested for subsequent planting. Mixture of QPM and normal grain during storage or sale will also decrease any potential benefit from the improved protein quality.

Finally, community level studies on QPM in human nutrition are still very much needed to quantify the effect of replacing conventional maize with QPM on the health and well-being of the potential beneficiaries. Researchers particularly need to pay sufficient attention to adequate

study design, statistical power, and control for confounding factors.

The development and dissemination of QPM as presented here is also relevant to micronutrient biofortification of food crops through genetic improvement of essential dietary vitamins and minerals. This is an important and growing area of research (Horton 2006) and many of the issues that have arisen or still remain after forty years of QPM research also apply to this area. The intervening factors that can affect the final nutritional or health benefits realized by target populations after the introduction of a micronutrient biofortified crop must be taken into account and, as with QPM, information and awareness will be important to achieve the greatest possible nutritional benefits. In addition, the targeting and impact assessment methodology and the data required for this methodology are likely to be similar among these nutritionally improved cultivars. The experiences and lessons learned from QPM could therefore inform efforts to develop, disseminate, and assess the impact of essential vitamin and mineral biofortified maize and other food crops.

Breeding and dissemination of QPM has made substantial progress in Africa. The breeding efforts were very productive, with commercial cultivars released in 17 countries. Agronomic characteristics of QPM cultivars are approaching those of conventional cultivars and new traits, such as resistance to diseases, have been incorporated. The increased protein quality has been clearly demonstrated in clinical studies elsewhere, but evidence from community level studies is still very limited and needs to be further developed in Africa. It is still early in the process to evaluate the adoption of QPM, but the cultivars are clearly popular in Ghana and Uganda, and the overall adoption in Africa is estimated at nearly 200,000 ha. To further steer QPM towards target farmers, more efforts are needed to understand the factors leading to its adoption or disadoption, in particular the agronomic characteristics and awareness of nutritional quality, but also the availability and cost of seed and ease of recycling.

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