REVIEW ARTICLE

Quality protein maize

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Maize (*Zea mays* L.) plays a very important role in human and animal nutrition in a number of developed and developing countries, worldwide. Breeding for improved protein quality in maize began in the mid-1960s with the discovery of mutants, such as *opaque-2*, that produce enhanced levels of lysine and tryptophan, the two amino acids deficient in maize endosperm proteins. However, adverse pleiotropic effects imposed severe constraints on successful exploitation of these mutants. Interdisciplinary and concerted research efforts led to amelioration of the negative features of the opaque phenotype, and the rebirth of 'Quality Protein Maize' (QPM). QPM holds superior nutritional and biological value and is essentially interchangeable with normal maize in cultivation and kernel phenotype. This paper deals with the salient sequence of events associated with the development of QPM, the present understanding of genetic, biochemical and molecular bases of QPM, and the recent technological developments that could potentially enhance the efficiency of QPM breeding and the reach of QPM cultivars.

THE nutritional well-being and health of all people are vital prerequisites for the development of societies. Significant advances have been made in genetic enhancement of crop plants for nutritional value. However, malnutrition still remains a widespread problem, and is particularly severe in developing countries with low per capita income. Globally, nearly 200 million children younger than five years are undernourished for protein, leading to a number of health problems, including stunted growth, weakened resistance to infection and impaired intellectual development. The intricate web of interconnections among nutrition, health, agriculture, environment, literacy, public policies and countless other factors, impose formidable challenges to the rapid improvement of the nutritional status of economically deprived sections of the society. Nevertheless, science and technology have been immensely aiding mankind's continuing efforts to combat poverty, hunger and malnutrition.

Maize is a major cereal crop for both livestock feed and human nutrition, worldwide. With its high content of carbohydrates, fats, proteins, some of the important vitamins and minerals, maize acquired a well-deserved reputation as a 'poor man's nutricerea Several million people, particularly in the developing countries, derive their protein and calorie requirements from maize. The maize grain accounts for about 15 to 56% of the total daily calories in diets of people in about 25 developing countries, particularly in Africa and Latin America[1], where animal protein is scarce and expensive and consequently, unavailable to a vast sector of the population. Cereal proteins, however, have poor nutritional value for monogastric animals, including humans, because of reduced content of essential amino acids such as lysine, tryptophan and threonine. Cereal proteins contain on an average about 2% lysine, which is less than one-half of the concentration recommended for human nutrition by the Food and Agriculture Organization (FAO) of the United Nations[2]. Therefore, healthy diets for humans and other monogastric animals must include alternate sources of lysine and tryptophan. From the human nutrition viewpoint, lysine is the most important limiting amino acid in the maize endosperm protein[3], followed by tryptophan[4]. The problem has been mainly dealt by supplementing grain with essential amino acids produced by bacterial fermentation. Although this approach works well for feeding animals, it is highly expensive. Besides, amino acids are often lost from foods processed from grain meals, as in the case of maize. For this and other reasons, it is valuable to adopt a genetic enhancement strategy in which essential amino acids are either incorporated or increased in grain proteins.

The need to genetically ameliorate the poor nutritional value of cereal grains such as maize has been recognized for a long time[5]. Realizing tangible achievements in improving nutritional quality of food crops through conventional breeding efforts necessitate long-term investments, sustained research efforts and patience, besides continuing administrative, financial and scientific support. It is in this context that the story of Quality Protein Maize (QPM) assumes significance, as it not only signifies a breeding achievement of enhancing grain protein quality in maize, but also highlights the spirit of scientific enquiry through painstaking research, and the ability to pursue 'hunches' against 'odds' through sustained and focused efforts.

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We shall try and trace this journey, firstly through a brief overview of some inherent factors responsible for the inadequate nutritional quality of maize endosperm protein.

Storage protein synthesis in maize endosperm

The maize kernel, like that of other cereal grains, includes pericarp (6%), endosperm (82%) and germ (12%)[6]. The main structural component of the endosperm is starch, a complex carbohydrate that constitutes on an average 71% of the grain and is a source of concentrated energy. Bulk of the proteins in a mature maize kernel is in the endosperm and germ; but, the germ protein is superior in both quantity and quality.

Zeins in the maize endosperm

The endosperm of maize contains a group of four structurally distinct alcohol-soluble proteins called 'zeins'[7–9], which are encoded by specific classes of structural genes that belong to a large gene family clustered in several genomic regions[10]. Their function is to store N, C and S and supply these important elements to the germinating seedling. Zeins have never been detected in any part of the plant other than the seed[11], wherein it is more abundant in the endosperm than in the embryo[12]. They form accretions called protein bodies in the rough endoplasmic reticulum of maize endosperm cells. Four types of zeins, α, β, γ and δ aggregate in a distinctive spatial pattern within the protein body. The study of zein synthesis is interesting, since it serves as a model system to analyse coordinated genetic regulation of several genes expressed at very high levels at a specific developmental stage[7,13].

In normal maize genotypes, zeins usually account for 50 to 70% of the endosperm protein and are characterized by a high content of glutamine, leucine and proline. Since zeins are essentially devoid of lysine and tryptophan[14], they dilute the contribution of these essential amino acids from the other types of endosperm proteins, which are collectively called 'non-zeins'. The non-zein fraction contains enzymes, structural polypeptides and membrane-associated proteins[15]. In normal maize, proportions of various endosperm storage protein fractions, on an average, are: albumins (3%), globulins (3%), zeins (60%) and glutelins (34%)[7]. Significantly, all fractions other than zeins are balanced in amino acid content and are quite rich in lysine and tryptophan. Suppression of lysine-deficient zein fraction without drastically altering the contribution of other fractions could be, thus, seen as a feasible approach to bring about improvements in the amino acid balance in maize grain.

Beginning of genetic manipulation of protein quality

Genetic variability for most traits in maize is incredibly high and amenable to enhancements. Attempts to improve protein content began towards the latter part of the nineteenth century. Prior to 1960s, efforts were rather limited to only screening elite maize germplasm and accessions to identify genotypes superior for this trait. Since no specific gene(s) conferring enhanced nutritional value was identified at that time, an improvement strategy involving recurrent selection could not be easily implemented. The lack of a simple genetic system also precluded the use of a straightforward back cross programme. Thus, protein quality remained more of a concern, with no immediate solutions in sight and no action-oriented strategies deployed to resolve the issue.

In the early 1960s, scientists manifested special interest in the search for gene mutants that could provide better quality protein in the maize endosperm. In 1963, researchers at Purdue University, USA, discovered that a mutation, designated *opaque-2* (*o2*), made grain proteins in the endosperm nearly twice as nutritious as those found in normal maize[16]. In fact, *o2* mutation was first described by Jones and Singleton in the early 1920s[17], but the nutritional significance of the mutation was first discovered by Mertz and coworkers[16,18]. This was soon followed by the discovery of another mutation, *floury-2* (*fl2*) that also has the ability to alter endosperm nutritional quality[19]. These mutations, which derive their names from soft, floury/opaque endosperm, alter the amino acid profile and composition of maize endosperm protein and result in two-fold increase in the levels of lysine and tryptophan in comparison with the normal genotypes. In addition, other amino acids such as histidine, arginine, aspartic acid and glycine show an increase, while a decline is observed for some amino acids such as glutamic acid, alanine and leucine. Decrease in leucine is considered particularly desirable as it makes leucine–isoleucine ratio more balanced, which in turn helps to liberate more tryptophan for niacin biosynthesis, and thus, helps to combat pellagra.

Early efforts and experiences in using *o2* cultivars

The discovery of 'high-lysine' mutations in maize aroused great optimism and considerable interest worldwide, as many believed that it would soon lead to development of nutritionally enhanced cereals. Breeding programmes were initiated in maize to develop inbred lines and populations using various endosperm quality mutants, mainly *o2*. In the initial stages, both *o2* and *fl2* genes were used singly or in combination with

each other. Later, as some undesirable effects of *fl2* mutant were discovered, its use slowed down and was discontinued.

For almost a decade, the major emphasis in most breeding programmes was on conversion of normal genotypes to *o2* mutant versions. Elite inbred lines making good hybrid combinations were converted as rapidly as possible through standard back cross approach. The *o2* composites and/or hybrids were experimentally tested and grown commercially in Brazil, Colombia, India, the United States, South Africa, Yugoslavia and Hungary during the late 1960s and early 1970s. However, the euphoria over the discovery of *o2* and its direct utilization in breeding programmes was soon tempered with the realization that pleiotropic effects of this mutation, namely a soft endosperm that results in damaged kernels, an increased susceptibility to pests and fungal diseases, inferior food processing and generally reduced yields, were not easily overcome[19]. In developing countries where farmers are accustomed to growing hard flints and dents, the kernel phenotype or appearance of the opaques was a major barrier to their acceptance.

The search then continued for new mutants that could alter the amino acid profile of maize endosperm proteins, by increasing the concentration of lysine and tryptophan. Among the additional mutants reported were *opaque-6*, *floury-3*, and *mucronate*[20,21]. Several of these mutants have been experimentally tried, but none offered any additional advantage over *o2* in maize breeding programmes, although they did improve our understanding about coordinate genetic regulation of storage protein synthesis in maize endosperm (discussed later).

As researchers and farmers became increasingly aware of the complex and interrelated problems associated with *o2* cultivars, the high hopes and optimism set forth earlier were greatly dampened, affecting resource funding and leading to a sharp decline in research efforts on enhancing nutritional value of maize grain. Only a few research centres and institutions, such as CIMMYT, Mexico; the University of Natal, South Africa, and the Crow's Hybrid Seed Company at Milford, Illinois, could sustain their efforts in improving the protein quality in maize.

Initial exploration of diverse options and strategies

The problems plaguing original, soft *o2* materials brought a turning point in the breeding efforts. Researchers at CIMMYT, Mexico, and at the University of Natal, South Africa, started to carefully examine the nature and seriousness of inherent problems, and came out with viable strategies to overcome the problems. This led to the recognition of '*o2* endosperm modifier genes' that alter the phenotype of *o2* mutants, giving them a normal hard (vitreous) appearance instead of a soft, chalky nature. Paez *et al.*[22] were the first to report on endosperm modification in *o2* kernels (50% translucent and 50% opaque). Subsequently, modified *o2* kernels with varying proportions of translucent and opaque fractions have been observed and studied by a number of workers[23–26]. While these genetic endosperm modifiers are difficult to work with due to their complex nature of inheritance[27], the strategy of selection for endosperm modification in *o2* background has been highly effective in ameliorating the negative features of the opaque phenotype.

Equally important was to think of some pertinent options and strategies that could be deployed effectively in developing germplasm competitive in agronomic performance and market acceptance. Most options fell into two broad categories; either involving or not involving specific 'high-lysine' mutants. The latter included options such as recurrent selection for improved protein quality, altering germ–endosperm ratio, and increasing aleurone layers of the maize grain. Developing high-lysine maize through recurrent selection in normal endosperm maize populations has been largely unsuccessful, although some researchers reported positive results[28–30]. In addition to the heavy dependence on laboratory facilities and the difficulty of transferring the high-lysine trait to other genetic backgrounds, this approach offered no assurance that the protein quality achieved would be biologically available.

Two interesting alternatives were considered: (i) exploiting double-mutant combinations; and (ii) simultaneous use of *o2* gene and the genetic modifiers of the *o2* locus. In several cases, the double-mutant combinations involving *o2* and other genes associated with endosperm quality were not always vitreous[31]. Although a double-mutant combination involving *o2* and *sugary-2* (*su2*) offered some advantages such as vitreous kernels, acceptable kernel appearance, lesser ear rot, increased lysine levels and better digestibility of protein[31,32], yield was severely affected due to the sum total of independent negative effects of the two mutations. Another double-mutant combination, *o2 fl2*, was also researched and not pursued further as modified vitreous kernels were encountered in only a few genetic backgrounds. The most successful and rewarding option involved a combined use of *o2* and the genetic modifiers of the opaque phenotype.

Development of quality protein maize – Sequence of salient events

Several factors contributed to the success of QPM research programme undertaken at CIMMYT. Undoubtedly, the most important factor had been an exemplary

interdisciplinary research strategy supported by appropriate methodology, and effective reorientation of research programme from time to time based on requirements. The QPM research team included breeders, biochemists, pathologists and entomologist, whose dedicated efforts led to the development of varieties and hybrids with grain type and yield comparable to the best conventional maize materials[33].

The guiding principle in developing competitive QPM genotypes was combining the nutritional advantages offered by the *o2* mutation with the *o2* modifiers that contribute to the genetically complex endosperm modification trait. To achieve this goal, a conservative approach was initially adopted in relation to biochemical characteristics, to strike a proper balance between protein content and quality. Since the *o2* gene boosts lysine levels two-fold, efforts were devoted to maintenance rather than further enhancing the levels of lysine at protein levels of 9–10% in the whole grain. This approach greatly facilitated breeding agronomically superior QPM genotypes, with a specific emphasis on alleviation of key problems related to grain texture of *o2* genotypes.

Segregation and analysis of kernels with a range of endosperm modification began at CIMMYT as early as in 1969 by John Lonnquist and V. L. Asnani. Modified kernels were classified into different categories and laboratory analyses were carried out to study the effects of the degree of modification on biochemical characteristics[34]. Modified ears were sorted from every possible source as they appeared during the conversion programmes and during the process of seed increase of *o2* maize materials. As expected, endosperm modification was very poor (often less than 25%), and the extent of modification varied significantly in different genetic backgrounds. Nevertheless, it was noticed that the Caribbean and Cuban flints, in general, tended to exhibit a higher frequency of modified kernels. A few *o2*-converted populations were then identified, which had unusually higher frequency of modified kernels. This provided considerable hope and enthusiasm, leading to a definite tilt towards the programme, and increased resource allocation for developing QPM germplasm.

Development of QPM donor stocks

Initial efforts towards development of QPM donor stocks with well-modified kernel phenotypes and good protein quality, proved to be not only difficult but also sometimes frustrating. Selection for kernel modification had to be practised at all stages, while simultaneously maintaining protein quality. Two approaches were effectively exploited in developing QPM donor stocks. The first was intrapopulation selection for genetic modifiers in *o2* backgrounds exhibiting a higher frequency of modified *o2* kernels. Four tropical populations [Composite K (H.E.*o2*), Ver.181-Ant.gp *o2** Venzuela-1 *o2*, Thai *o2* Composite, PD (MS6) H.E.*o2*] and one highland population [Composite I] which met this criterion were chosen for this approach. Controlled full-sib pollination was employed in the initial cycle followed by modified ear-to-row system suggested by Lonnquist[35]. Selection was practised for modified ears and modified kernels at all stages[19]. The second approach involved recombination of superior hard endosperm *o2* families. The yellow and white families were recombined separately to develop 'Yellow H.E.*o2*' (yellow, hard endosperm *o2*) composite and 'White H.E.*o2*' composite, respectively. Selection of modified ears, showing high frequencies of modified kernels with good protein quality, was practised for 3–4 cycles. By the mid-1970s, a high degree of endosperm modification was achieved in these materials (Figure 1 *a* and *b*), and the genotypes were ready for utilization as QPM donor stocks.

Conversion of non-QPM materials into QPM versions

Development of QPM donor stocks was followed by large-scale QPM germplasm development efforts in a

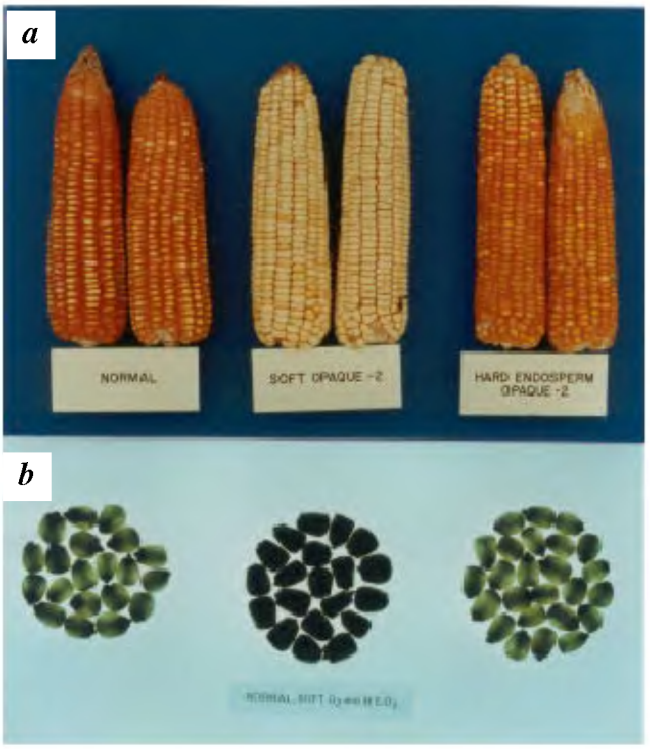

Figure 1. QPM (H.E.*o2*) ears showing a hard, vitreous kernel phenotype comparable to that of normal maize. ***a***, Soft, chalky texture of the *o2* kernels without the endosperm modifiers; ***b***, Backlit kernels from normal, soft *o2* and QPM (H.E.*o2*) ears, illustrating the extent of endosperm modification in the QPM kernels.

wide array of genetic backgrounds, representing tropical, subtropical and highland maize germplasm, and involving different maturities as well as grain colour and texture. Owing to the complexity and nature of kernel modification trait, it was realized in the beginning that a standard back cross programme might not work. Therefore, an innovative breeding procedure, designated as 'modified back crossing-cum-recurrent selection', was designed to efficiently handle the conversion programme as rapidly as possible[31,36]. A number of advanced maize populations in CIMMYT maize programme were successfully converted to QPM populations using this procedure. During conversion, emphasis was placed on yield, kernel modification and appearance, reduced ear rot incidence, rapid drying and other desirable agronomic attributes. Besides the conversion programme, considerable resource allocation and research efforts were also devoted to the development of broad-based QPM gene pools[19].

Emphasis on QPM germplasm management

In a period extending over 5–6 years, a huge volume of QPM germplasm was developed that could meet the needs of several production environments in the tropical and subtropical areas. The yield gap was narrowed down significantly, with concomitant increase in the average kernel modification scores of the QPM ears. No differences were encountered in normal and QPM counterparts for moisture content, and incidence of ear rot or stored grain insect pests. During all the stages of improvement, grain protein quality was monitored and effectively maintained[34,37].

It was then considered important to devise a suitable strategy that permits systematic and efficient improvement and utilization of this valuable germplasm. Merging, consolidation and reorganization of QPM germplasm were thus attempted. Knowledge of the germplasm in terms of agronomic performance and biochemical characteristics, besides grain colour and maturity, were the principal guiding factors in this merging process. Handling of QPM materials in homozygous *o2* background was emphasized at this point, to facilitate faster progress and rapid accumulation of favourable modifiers for kernel modification, weight and density. Working on homozygous *o2* backgrounds had the additional advantage of reducing errors that generally occur due to misclassification and selection of non-QPM kernels in the segregating generations. Thus, several tropical and subtropical QPM gene pools were formed (Table 1). Simultaneously, 10 QPM advanced populations (six tropical and four subtropical) were identified and tested through International Progeny Testing Trials for further dissemination and improvement by the National Agricultural Research System (NARS) in diverse countries. Handling of QPM germplasm, including pools and populations, has been discussed in earlier reports[19,36,37].

Thrust on QPM hybrid development

An initiative on QPM hybrid breeding at CIMMYT was made in 1985, as the QPM hybrids offered several advantages in relation to (a) exploitation of heterosis; (b) ease in maintaining seed purity in contrast to open-pollinated QPM cultivars; (c) uniformity and stability in kernel modification in hybrids, and (d) requirement for minimum protein quality monitoring as long as the purity of parental lines is ensured. The last point is particularly important since not many developing countries have well-established laboratories to analyse protein quality.

Table 1. CIMMYT QPM gene pools and their characteristics

QPM pool no.	Adaptation	Maturity	Seed colour	Seed texture	Kernel quality characteristics*			Quality index
					% protein	% lysine	% tryptophan	
Pool 15 QPM	Tropical	Early	White	Flint–Dent	9.1	4.2	0.94	4.6
Pool 17 QPM	Tropical	Early	Yellow	Flint	8.9	4.5	1.04	4.5
Pool 18 QPM	Tropical	Early	Yellow	Dent	9.9	4.0	0.93	4.6
Pool 23 QPM	Tropical	Late	White	Flint	9.1	3.8	1.03	4.2
Pool 24 QPM	Tropical	Late	White	Dent	9.4	3.8	0.92	4.0
Pool 25 QPM	Tropical	Late	Yellow	Flint	9.8	4.0	0.94	4.0
Pool 26 QPM	Tropical	Late	Yellow	Dent	9.5	4.1	0.90	4.3
Pool 27 QPM	Subtropical	Early	White	Flint–Dent	9.5	4.2	1.05	4.8
Pool 29 QPM	Subtropical	Early	Yellow	Flint–Dent	9.2	4.3	1.06	4.8
Pool 31 QPM	Subtropical	Medium	White	Flint	10.2	4.1	0.96	4.5
Pool 32 QPM	Subtropical	Medium	White	Dent	8.9	4.2	1.04	4.5
Pool 33 QPM	Subtropical	Medium	Yellow	Flint	9.3	–	1.05	4.2
Pool 34 QPM	Subtropical	Medium	Yellow	Dent	9.1	4.1	1.10	4.5

*Per cent lysine and tryptophan content in grain protein (source: S. K. Vasal).

Table 2. Performance of some superior CIMMYT QPM hybrids in international trials[a]

Pedigree	Yield (t/ha)	Days to silking	Endosperm hardness[b]	Tryptophan content[c]	Ear rot (%)
A. Group-I					
CML142 X CML146	6.48	55	2.0	1.00	3.7
CML159 X CML144	6.39	56	1.6	1.00	4.3
CML145 X CML144	5.81	54	2.0	0.84	5.8
CML158 X CML144	5.59	55	1.3	1.00	7.1
CML146 X CML150	5.48	56	3.6	0.80	8.7
Normal hybrid check	5.58	56	2.0	0.70	9.5
B. Group-II					
CML142 X CML186	9.56	75	1.9	0.90	4.7
CML176 X CML142	9.36	80	1.7	0.90	4.9
CML186 X CML149	9.21	76	1.8	0.90	5.7
CML173 X CML142	8.99	74	1.8	1.00	4.9
Normal hybrid check	8.51	78	2.0	0.60	5.8

[a]Group-I: White QPM hybrids tested across 15 locations in El Salvador, Guatemala and Mexico in 1998; Group-II: Subtropical QPM hybrids across 23 test locations in Latin America, Asia and Africa during 1977–1999 (source: S. K. Vasal).
[b]Endosperm modification score on a scale of 1 (completely vitreous) to 5 (completely opaque).
[c]Per cent tryptophan content in grain protein.

Analysis of combining ability in the QPM germplasm resulted in identification of potential parental lines in QPM hybrid breeding[38,39]. Concurrently, inbred line development efforts have been strengthened. Several QPM hybrid combinations were derived and tested in international testing programme at multiple locations in Asia, Africa and Latin America (Table 2). Some of the QPM hybrids performed equal to or better than some of the local checks included in the trials. The encouraging performance of QPM hybrids in various countries stimulated intensive efforts, particularly in the last decade, to derive superior hybrid combinations.

Simultaneous to the development of QPM germplasm, some research groups focused their attention on understanding of the genetic, biochemical and molecular basis of zein synthesis and the role of high-lysine loci such as *o2* and others. Particularly praiseworthy, in this context, were the contributions of a research team led by Brian Larkins at the University of Arizona, USA. Development of QPM germplasm further enhanced the interest of researchers in establishing how the modification of endosperm is influenced, both at the biochemical and molecular levels.

Genetic, biochemical and molecular analyses of QPM

Regulation of zein synthesis in endosperm

The high-lysine loci in maize play a vital role in the coordinate expression of different zein gene families, which may even be located far apart in the genome. The role of these genes in the control and biosynthesis of seed storage proteins in maize has been examined by several researchers[40–42]. The *o2* mutant differentially regulates and reduces zein gene transcription, particularly that of the most abundant α-zeins[43]. The *fl2* mutation appears to correspond to a defective α-zein protein whose signal polypeptide is not cleaved and shows a general reduction in all four types of zeins[27,42].

The *Opaque-2* (*O2*) gene was cloned using a transposon tagging strategy with the maize mobile genetic elements, *Spm*[44] and *Ac*[45]. The *O2* gene encodes a transcription factor required mainly for the expression of 22 kDa α-zein-coding genes and a 32-kDa albumin gene *b-32*, and is necessary for their expression[43,46–49]. The *O2* protein contains a basic domain/leucine zipper (bZIP) motif identified in DNA-binding proteins of animal proto-oncogenes and in transcriptional regulators of yeast[46]. Lower α-zein content in *o2* endosperm results in protein bodies that are about one-fifth to one-tenth the normal size; this, in turn, is presumed to alter packing of starch grains during seed desiccation, thereby conferring a characteristic soft texture to the kernel. With the reduction of α-zeins in the endosperm due to *o2* mutation, there is an usually concomitant increase in the level of γ-zeins[50]. Transcription factors of bZIP type frequently function as heterodimers. Heterodimerizaton between *O2* and another bZIP protein, OHP1, has been demonstrated[51], suggesting the involvement of multiple bZIP proteins in transcriptional control of zein genes[52].

Analyses of endosperm modification

Analysis of inheritance of modified endosperm in diverse *o2* backgrounds indicated gene dosage effects on kernel texture, besides incomplete and unstable pene-

trance of the endosperm modifier genes[53]. Glover and Mertz[40] also indicated that the modified endosperm texture is polygenically controlled with additive type of genetic variation playing an important role, although in some materials a few major genes may contribute significantly to kernel modification. The genetic background of the material and its kernel texture could also influence kernel modification and frequencies of various modification classes. Phenotypic variation ranging from completely unmodified to completely modified kernels was observed in single ears of F_2 progenies segregating for kernel modification[54]. Occurrence of such a wide range of segregants in single ears suggested that the number of independent genetic factors responsible for endosperm modification might not be very high.

The mechanism(s) by which the modifier genes convert the starchy endosperm of *o2* to a normal phenotype is still poorly understood, but some important clues have been obtained through analysis of biochemical changes in modified *o2* endosperm. QPM genotypes appear to have levels of α-zein comparable to unmodified *o2* lines, but the level of γ-zein is increased 2–3 fold. The role of γ-zeins in conversion of an opaque seed to a vitreous phenotype is still not fully understood. One possibility is cross-linkages of the protein through disulphide bridges[54,55]. The increased γ-zein in QPM has been found to be the result of elevated steady-state levels of γ-zein mRNA[56]. By discovering the specific effects of *o2* modifiers on the accumulation of γ-zein protein and RNA, Or and coworkers[52] suggested that the products of the modifier genes interact with γ-zein mRNA transcripts and enhance their transport from the nucleus or increase their stability and translation. Lopes and Larkins[57] also hypothesized that the *o2* modifier gene near the telomere of chromosome 7L encodes a trans-acting factor that affects γ-zein RNA stability.

Significant progress has been made in recent years in the identification of the number and location of major genetic loci possibly responsible for endosperm modification. Analysis of segregating progenies[54,57] and Recombinant Inbred Lines (RILs)[57] derived from crosses between *o2* and modified *o2* genotypes indicated two independent loci affecting seed opacity and density. Consistent association between endosperm modification and enhanced accumulation of the γ-zeins[57] also suggested that either the γ-zeins are directly involved in the process of seed modification or the modifier gene(s) could be tightly linked to those responsible for γ-zein synthesis. Two major loci involved in *o2* modification have been pointed out by RFLP (Restriction Fragment Length Polymorphism) analysis; one locus maps near the centromere of chromosome 7 and the second maps near the telomere on the long arm of chromosome 7 (ref. 58). *opaque-15*, a mutation that maps near the telomere of chromosome 7L, appears to have the properties of a defective *o2* modifier[59]. Variation with respect to endosperm modification may also arise from several minor modifier loci, though the number and possible effects of such loci are yet to be understood.

Information available so far strongly suggests the occurrence of two to three major loci influencing endosperm modification from the opaque to vitreous phenotype, with several minor factors possibly fine-tuning the process. This enhances the possibility of selecting lines with reasonable levels of endosperm modification, while retaining the nutritional value of *o2*. Availability of a combination of molecular probes that would allow selection of endosperm modifiers in *o2* genotypes, prior to selection for agronomic characteristics, shall facilitate rapid and efficient conversion of non-QPM inbred lines into QPM counterparts. Recently, Lin *et al.*[60] identified some DNA-based markers that could be of value in selection of endosperm modifiers contributing to the QPM phenotype. The utility of three maize microsatellite markers that are *o2* gene-specific (phi057, phi112 and umc1066) in molecular-marker assisted selection for *o2* is currently being explored in the QPM breeding programme at CIMMYT, Mexico.

'Nutritional genomics'[61] has the potential to further enhance the nutritional value of grain crops like maize through elucidation and effective manipulation of biochemical pathways and molecular mechanisms controlling kernel quality. Genomic techniques are currently being employed by some research groups in the developed countries, to investigate the patterns of gene expression in mutants influencing maize kernel texture. By monitoring the influence of genetic endosperm modifiers on the patterns of gene expression in mutants such as *o2* and *fl2*, mechanism(s) underlying restoration of normal kernel texture may be better understood. Such knowledge could be of considerable value in improving the precision and efficiency of QPM breeding.

Modification of genes encoding zeins[62] and genetic engineering of key enzymes involved in the lysine biosynthetic pathway, namely aspartate kinase and dihydropicolinate synthase[63], are some of the other alternatives to enhance the nutritional value of maize grain by increasing the lysine content. Deregulation of lysine biosynthetic pathway via genetic engineering may prove to be effective, provided there is no impairment of normal metabolic functions in the vegetative tissues, and the increased lysine is confined to the kernel.

Development of rapid and sensitive assays for protein quality

Rapid and reliable determination of lysine content is one of the major limiting factors for QPM breeding programmes, worldwide. Lysine measurements made by conventional amino acid analysis are expensive and slow, making them prohibitive for most breeding pro-

grammes. Therefore, such programmes have traditionally relied on indirect measurement of lysine based on colorimetric analysis[34,64,65] or by indirectly inferring lysine content through colorimetric analysis of tryptophan content[34]. However, colorimetric methods are not precise and have many limitations[66]. A breakthrough was made in 1990s through the development of an enzyme-linked immunosorbent assay (ELISA) that provided a more objective and rapid means of estimating lysine content in maize endosperm. The genesis of this assay is as follows.

The origin of lysine-containing proteins in cereal grains is usually determined by extracting the flour with different solvents. SDS-PAGE of these fractions revealed that the majority of the non-zein proteins and nearly 80% of lysine in the endosperm proteins were recovered in the soluble protein fraction[50]. Habben and coworkers[50] made a complex antiserum against the soluble protein fraction and used it in an ELISA to estimate the level of non-zein proteins in the normal and *o2* endosperm. Although the correlation between lysine and non-zein content was found to be high ($r^2 = 0.5$), the analysis suggested that specific lysine-rich proteins in the non-zein fraction may be responsible for much of the variability in lysine content of maize endosperm.

From the analysis of cDNA clones, a gene-coding elongation factor-1α (EF-1α), whose synthesis is significantly increased in *o2* endosperm, has been identified[15]. EF-1α is a lysine-rich protein (10% lysine) that is highly abundant in eukaryotic cells and appears to be involved in multiple cellular processes[67,68]. Habben *et al.*[15] developed an ELISA using EF-1α antiserum, to measure the level of this protein in maize genotypes. The study revealed a remarkably high positive correlation ($r^2 = 0.92$) between lysine levels in the endosperm and EF-1α content. The maize EF-1α has been recently characterized and its relationship to protein quality in the endosperm demonstrated[69].

Concentration of EF-1α, thus, appears to provide a useful index of the lysine content in the cereal grain proteins. The ELISA for EF-1α provides a sensitive, efficient, less laborious, and inexpensive method of monitoring the lysine content of maize grain, and is more amenable to automation than non-zein quantification[70]. Several countries in the QPM Research and Development Network facilitated by CIMMYT, have begun to utilize the ELISA in rapid screening of QPM breeding materials for protein quality.

Nutritional superiority and biological value of QPM

The nutritional benefits of QPM for people who depend on maize for their energy and protein intake, and for other nutrients, are indeed quite significant. Mertz *et al.*[16] first reported that the lysine content in *o2* was 3.3 to 4.0 g per 100 g of endosperm protein, which was more than twice that of normal maize endosperm (1.3 g lysine/100 g endosperm protein). Several researchers later demonstrated the superior protein quality and protein digestibility of QPM over normal maize[71–73]. The studies indicated that the QPM protein contains, in general, 55% more tryptophan, 30% more lysine and 38% less leucine than that of normal maize.

Besides protein quality, another important factor is 'biological value', which refers to the amount of absorbed nitrogen needed to provide the necessary amino acids for different metabolic functions. The biological value of normal maize protein is 45%, while that of *o2* maize is 80%. Only 37% of common maize protein intake is utilized compared to 74% of the same amount of *o2* maize protein. A minimum daily intake of approximately 125 g of *o2* maize might guarantee nitrogen equilibrium. This could not be obtained by using even twice the amount of normal maize. The nitrogen balance index for skim milk and *o2* maize protein is 0.80 and 0.72, respectively, which indicates that the protein quality of QPM is 90% of that of milk. Besides, around 24 g of normal maize per kg of body weight is required for nitrogen equilibrium, compared to only around 8 g for QPM[72,73].

The other nutritional benefits of QPM include higher niacin availability due to a higher tryptophan and lower leucine content, higher calcium and carbohydrate[73], and carotene utilization[74]. Further, high quality protein maize can be transformed into edible products without deterioration of its quality or acceptability, and can be used in conventional and new food products. Graham *et al.*[75] stated that '*To anyone familiar with the nutritional problems of weaned infants and small children in the developing countries of the world, and with the fact that millions of them depend on maize for most of their dietary energy, nitrogen and essential amino acids, the potential advantages of quality protein maize are enormous. To assume that these children will always be given a complementary source of nitrogen and amino acids is a cruel delusion*'.

The nutritional and biological superiority of QPM has also been amply demonstrated in model systems such as rats[76], pigs[77,78], infants and small children[73,79] as well as adults[80]. In Guatemala, it was demonstrated that *o2* maize has 90% of the nutritive value of milk protein in young children. Children in Colombia suffering from Kwashiorkar, a severe protein deficiency disease, were brought back to normalcy on a diet containing only *o2* maize as the source of protein. QPM would have equally beneficial effects on adults, as in case of infants and children[80].

Besides its obvious significance in human health, QPM could play an increasingly important role in reducing the protein supplement in animal feed, if used as

a gradient. Gevers[81] indicated the potential utility of high-lysine maize in feeds for monogastric animals, and how QPM could bring in significant immediate rewards through direct industrial exploitation. QPM can also be used as an ingredient in the preparation of composite flours to supplement wheat flour for bread and biscuit preparation. Composite flours (10% maize flour) are used commercially in sub-Saharan countries such as Zambia, Zimbabwe and Ghana. Brazil also uses composite wheat flours utilizing cassava and maize flours.

Renewed emphasis on QPM R&D in some national programmes

Since the mid-1990s, QPM was tested at multiple research stations all over the world, with encouraging results. This involved testing between 600 and 1000 hybrid combinations of maize per year, a phenomenal research effort. QPM acreage is expected to be around one million hectares (2.5 million acres) spread over 20 countries. Data from 32 locations across Africa, Asia, and Latin America show that QPM hybrids are capable of outperforming current commercial hybrids by an average of 10%. It is heartening to note that QPM is transforming agriculture in some of the poorest parts of countries such as in China, Mexico, Ghana and Peru.

The R&D efforts on QPM have greatly benefited from the generous support of Sasakawa Global 2000, the Nippon Foundation and the UNDP. Successful utilization of a QPM variety 'Obtanpa' (meaning 'good mother') in Ghana, released in 1992, is particularly noteworthy. Nearly 50% of the area in the country is currently planted to this variety. Recently, several

Table 3. Some recent QPM varietal releases in Latin America, Africa and Asia

Name	Type	Pedigree	Country
HQ INTA-993	Hybrid	(CML144 X CML159) CML176	Nicaragua
NB-Nutrinta	OPV[a]	Poza Rica 8763	Nicaragua
HB-Proticta	Hybrid	(CML144 X CML159) CML176	Guatemala
HQ-61	Hybrid	(CML144 X CML159) CML176	El Salvador
HQ-31	Hybrid	(CML144 X CML159) CML176	Honduras
ICA	Hybrid	(CML144 X CML159) CML176	Colombia
INIA	Hybrid	CML161 X CML165	Peru
FONAIAP	Hybrid	(CML144 X CML159) CML176	Venezuela
BR-473	OPV	–	Brazil
BR-451	OPV	–	Brazil
Assum Preto	OPV	–	Brazil
441C	Hybrid	CML142 X CML176	Mexico
H-551C	Hybrid	CML142 X CML150	Mexico
H-553C	Hybrid	(CML142 X CML150) CML176	Mexico
H-519C	Hybrid	(CML144 X CML159) CML170	Mexico
H-368EC	Hybrid	CML186 X CML149	Mexico
H-369EC	Hybrid	CML176 X CML186	Mexico
VS-537C	OPV	Poza Rica 8763	Mexico
VS-538C	OPV	Across 8762	Mexico
Susuma	OPV	Across 8363SR	Mozambique
Obatampa	OPV	Across 8363SR	Mali
Obangaina	OPV	Across 8363SR	Uganda
Obatampa	OPV	Across 8363SR	Benin
Obatampa	OPV	Across 8363SR	Burkina Faso
Obatampa	OPV	Across 8363SR	Guinea
GH-132-28	Hybrid	P62, P63	Ghana
QS-7705	Hybrid	–	South Africa
Zhong Dan 9409	Hybrid	Pool 33 X Temp. QPM	China
Zhong Dan 3850	Hybrid	–	China
Quian 2609	Hybrid	Tai 19/02 X CML171	China
Yun Yao 19	Hybrid	(CML140)	China
Yun You 167	Hybrid	(CML194)	China
Lu Dan 206	Hybrid	(P70)	China
Lu Dan 207	Hybrid	(P70)	China
Lu Dan 807	Hybrid	(P70)	China
Hybrid 2075	Hybrid	(CIMMYT QPM Populations)	China
Shaktiman-1	Hybrid	(CML142 X CML150) CML176	India
Shaktiman-2	Hybrid	CML176 X CML186	India
HQ-2000	Hybrid	CML161 X CML165	Vietnam

[a]OPV, open pollinated variety.

single- and three-way cross hybrids such as GH132-88, GH110-81 and GH2823-88 have been developed, which are superior in grain yields to 'Obtanpa'[82]. Several potential commercial channels for QPM utilization in Ghana have also been identified, including infant and institutional child-feeding programmes, poultry and piggery. Beginning in 1982, South Africa pursued vigorously the development of modified *o2* maize hybrids, leading to the development of several hybrids such as HL1, HL2 and HL8, possessing hard endosperm, good yield potential and tolerance to diseases[83].

QPM interest in Mexico has grown with the support and commitment of the Mexican government, to cover substantial area under QPM hybrids in a short span. Particularly in the last 3–4 years, INIFAP, Mexico, had developed and released several QPM hybrids and composites in collaboration with CIMMYT (Table 3). A few central and Latin American countries have also either released or are ready to release QPM hybrids. The Brazilian maize programme gave considerable emphasis to QPM[84], and has commercialized two QPM cultivars, BR-451 and BR-473. QPM hybrid breeding in Brazil has been greatly strengthened and several promising hybrids are in the pipeline.

China leads the Asian maize-growing countries in demonstrating the potential and impact of QPM. 'Zhong Dan 206', the first high-lysine single-cross hybrid with soft endosperm, was released for commercial use in 1988. More than 100,000 hectares are currently planted to QPM hybrids. It is expected that more than 30% of the total maize-growing area in China will be covered by QPM hybrids by 2020 (ref. 85). The Chinese government has shown great commitment to promote QPM hybrids all over the country, and QPM R&D efforts are being taken up by several academies all across the country. Most notably, in the Guizhou Province (southern China), one of the poorest provinces in China, QPM intervention is being effectively used to alleviate poverty and to improve the nutritional and economic well-being of the farmers[86].

Several countries in Asia, Africa and Latin America, such as India, China, Honduras, Bolivia, Colombia, Ethiopia, Mozambique, Tanzania, Uganda, Zimbabwe and South Africa, are part of QPM Research and Development Network facilitated by CIMMYT, for the improvement and promotion of QPM in developing countries. Several of these countries have been developing, testing and disseminating QPM cultivars. The network is laying particular emphasis on conducting on-farm trials, strip tests and 'QPM Field Days', to demonstrate the benefits of QPM to the farmers and end-users.

QPM R&D efforts in India

Over 85% of the maize produced in India is currently used for human consumption, particularly in the economically deprived areas where protein malnutrition and hunger are apparent. India was among the first countries in the world to focus on improvement of maize quality soon after the nutritional benefits of *o2* mutation were brought to light in 1964. As a result of a research programme initiated in 1966 under the All-India Coordinated Maize Improvement Project (AICMIP), three *o2* composites, namely Shakti, Rattan and Protina were developed and commercially released in 1970 (ref. 87). Due to major constraints in agronomic performance and kernel phenotype of these cultivars, another research programme was initiated in 1971, for developing high-yielding *o2* materials with hard endosperm, by utilizing suitable germplasm from both India and abroad[88]. This led to the development of a modified, nutritionally superior *o2* composite in 1997, designated as 'Shakti-1'.

Since 1998, intensive efforts have begun at various centres in the country under the National Agricultural Technology Project (NATP). These efforts resulted in the recent release of two QPM hybrids, 'Shaktimaan-1' (a three-way cross hybrid) and 'Shaktimaan-2' (a single-cross hybrid), with the CIMMYT QPM inbreds as parental lines (Table 3); these hybrids are particularly suitable for cultivation in the state of Bihar. The current thrust is effective utilization of QPM and its products in diversified ways, by conversion into a variety of products for use as infant food, health food/mixes, convenience foods, specialty foods and emergency ration[89]. It is envisioned that apart from the possible impact on the health status of malnourished segments of the society, QPM can also be a source of rural entrepreneurship, as several QPM-based food products can be easily prepared in the villages.

Concluding remarks

The strategy used by the CIMMYT researchers in developing QPM has proved to be successful. Practically all QPM research programmes in different countries are now using this approach based on the combined use of the *o2* gene and the endosperm modifiers. The award of the prestigious 'World Food Prize' in the year 2000 to Surinder K. Vasal and Evangelina Villegas is recognition of an outstanding example of interdisciplinary team work of the CIMMYT researchers, and signifies the relevance of QPM to millions of people across the world. QPM is now of major interest to breeders, geneticists, seed producers and the industry, as its large-scale production promises to offer significant benefits. The challenge now is how to effectively disseminate the technology in the needy areas, specifically in the developing countries in Asia, Africa and Latin America[90], where maize plays a prominent role in human and animal nutrition.

It is encouraging to note that several national maize programmes are placing major thrust on QPM cultivar development based on identified local needs. Hybrid development efforts in QPM have also progressed considerably. It is hoped that many countries now involved in the QPM network will be able to select some of the most promising hybrid combinations developed by CIMMYT for release in respective countries in the near future. Simultaneously, efforts are being made by the NARS for conversion of elite, local inbred lines into QPM versions by effectively making use of donor stocks, particularly those developed by CIMMYT. But, there are still some practical problems to overcome for widespread development and deployment of QPM cultivars, particularly in the developing countries[91]. In a vast majority of these countries, a large proportion of maize is produced by small farmers, who use the grain mainly for their own consumption or save the seed for subsequent sowings. Introduction and effective exploitation of high-yielding QPM hybrids may be difficult in such areas. Nevertheless, concerted efforts by public sector institutions can lead to significant increase in QPM hybrid adoption, even under the small-farm situations. This optimism is based on successful deployment of QPM hybrids in African countries such as Ghana. There are other constraints to overcome, such as lack of adequate funding, trained scientific and technical personnel, and more importantly, knowledge of the possible benefits to be derived from high quality protein maize. Dedicated efforts are required for better public awareness and dissemination of QPM technology, particularly in economically deprived regions where maize is used for food and feed purposes, and complementary sources of proteins are either scarce or unaffordable.

In the coming years, there will be an increasing application of molecular genetic tools in QPM research and development. Research programmes at some institutions such as CIMMYT, Mexico, Texas A&M University, USA, EMBRAPA, Brazil, and University of Natal, South Africa, are already making use of the new bioscience tools and technologies on a limited scale. Greater understanding and control over mechanism(s) controlling kernel modification are vital to increase the pace of progress in QPM germplasm and cultivar development in diverse genetic backgrounds. This would essentially require more intensive studies in relation to the role of γ-zein proteins in enhancing lysine content, better characterization of the zein proteins, molecular tagging of endosperm modifier gene loci and isolation of the modifier genes themselves. With the power of genomic technologies now available, and likely to be further developed, it is possible to effectively complement the breeding efforts, provide a greater thrust to the QPM technology and derive significant nutritional and economic benefits for the society.

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Received 9 July 2001; revised accepted 19 September 2001