emergent
Life Sciences Research

Review Article

Breeding strategies for quality protein maize: A Review

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Abstract

Zein proteins in maize seeds regulate food and feed quality and belong to several subfamilies. Most maize prolamin is α-zeins, which are rich in glutamine, leucine, and proline but lacking in critical amino acids (lysine and tryptophan). RNAi experiments show that 22kD zeins downregulate the quality protein maize (QPM) phenotype, not 19kD. The opaque-2 mutation caused a chalky, soft endosperm and many defects. QPM genotypes with opaque-2 alleles and firm kernels were improved. As opaque-2 is recessive and biochemical lysine and tryptophan analysis is costly, conventional backcrossing alone is not very useful. Marker-assisted selection (MAS) allowed top inbreds to be homozygous *o2o2* utilizing opaque-2 gene-specific markers. Vivek QPM-9, a mixture of two QPM introgression lines, benefits various clients and customers. This review highlights the breeding strategies for QPM.

Keywords introgression, marker-assisted selection, opaque-2, quality protein maize

Received: 09 November 2023
Accepted: 13 January 2024
Online: 19 January 2024

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Emer Life Sci Res (2024) 10(1): 19-32

E-ISSN: 2395-6658
P-ISSN: 2395-664X

DOI: https://doi.org/10.31783/elsr.2024.1011932

Introduction

Maize as a cereal crop has contributed very significantly to the development of human society, after wheat and rice. Maize (*Zea mays* L.) is the most important cereal crop in the world. India and other emerging nations utilize maize as a staple crop, including Africa, Mesoamerica, and West Africa [1]. It provides almost 20% of the calories eaten in Africa and Mesoamerica [2], but the amount of maize eaten varies widely from one region to the next [3]. For instance, Mesoamericans eat 60% more maize than Andean people, just 4% more than those in South Asia. While it is a key source of protein, accounting for as much as 70% of protein consumption in underdeveloped nations. Over 405 billion people in poor nations get over 30% of their caloric intake from maize, wheat, and rice. Notably, more than 170 nations cultivate an annual total of 193.7 M hectares, yielding an average of 5.75 t/ha, or 1147.7 M MT of maize [4]. With maize output of 27.8 mt, a cultivated area of 9.2 m ha, and a productivity of 2.9 t per hectare. India is in the top 10 among nations that cultivate maize. Only 4% of total maize land is used for growing this crop, although it accounts for 2% of total output. Among other things, the feed, starch, and biofuel industries use 83% of the world's maize harvest. 60% feed > 22% industries > 17% food are the most common ways that maize is used across the world making it the world's most important industrial crops [5]. In addition, maize provides a rich source of raw materials for

the production of over three thousand different products, opening up several avenues for value addition, and making it a key factor in agriculture across the world.

It is a crucial crop, especially for the underprivileged of the world's poorest nations for meeting food requirements and contributing to food and nutritional security. However, the amino acids lysine and tryptophan, both of which the human body cannot produce, are missing in maize. Improved nutrition is essential for every society to flourish. Malnutrition is still a major issue, especially in underdeveloped nations. Globally, about 200 million children are under-nourished to meet the requirements of protein, leading to many health concerns. Due to the intricate network of interdependencies between agriculture, health, environment, literacy, nutrition, public policies, and many other factors, rapid improvement in the nutritional status of economically disadvantaged segments of society is a formidable challenge [5]. Though other foods like eggs, meat, dairy, and legumes can be sources of these amino acids, these are prohibitively expensive or non-accessible for those living in rural parts of impoverished nations [6]. Due to financial constraints and economic status, they are unable to purchase certain food items. Consuming maize regularly may induce protein insufficiency and a host of malnutrition symptoms including diarrhea, gastrointestinal issues, stunted development, edema, and more. Kwashiorkor is the worst of the diseases, and it affects other symptoms as well [7]. Pellagra was brought on by a lack of niacin, which may be remedied by eating maize [8]. Dermatitis, dementia, inflammation of the tongue, skin lesions, and weakness are all signs of Pellagra illness. The recommended daily allowance of lysine for humans is 5 mg although the average serving of cereal only provides 1.5 to 2 percent. A lack of lysine and tryptophan in normal maize is the root cause of many diseases. Quality protein maize (QPM) has around twice as much tryptophan (0.5-1.1 g/100 g of endosperm protein) and lysine (2.7-4.5 g/100 g) as compared to normal corn [9]. The differences between normal maize and QPM are explained in Table 1 and Figure 1.

Storage proteins of maize

The mature maize kernel consists of an embryo (10%), endosperm (85%), and pericarp (5%): indicating that it is mostly made up of endosperm. While protein content in maize embryos is 18% and the oil proposition is 30%, with high quality and quantity. Protein makes up just 10% of the endosperm, whereas starch, a kind of carbohydrate constitutes the vast majority (90%). These endosperm cells create two distinct regions i.e. vitreous endosperm and starch endosperm, with the former transmitting light, the other region comprising starchy endosperm does not transmit light,

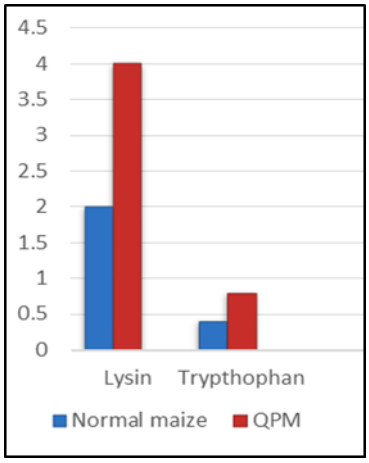

Figure 1. Graphical representation of lysine and tryptophan in normal maize (NM) and quality protein maize (QPM)

Table 1. Comparison of normal maize with QPM (22 and 16)

Parameters	Normal maize	QPM
Protein contents	>8%	>8%
Lysine content	1.6 - 2.6 g/100gms	2.7 – 4.5g/100gms
Tryptophan	0.2 -0.6 g/100gms (0.4)	0.5 – 1.1 g/100gms (0.8)
Biological value	~45%	~80%
Protein intake utilization	37%	74%
Maize required for N equilibrium/kg of body wt	24g	8g
Other benefits	-	Higher Ca, carbohydrates, carotene, niacin than NM

and appears dark color [10]. Maize endosperm has two types of proteins- zein and non-zein proteins and zein proteins are mainly prolamines (> 60%), which are soluble in alcohol [11]. On the other hand, non-zein proteins are of three types- albumins (3%) soluble in water, globulins (3%) soluble in salt, and glutens (34%) soluble in alkali. Further zein proteins are made up of four different polypeptide protein bodies viz., alpha, beta, gamma, and delta zein proteins. Among these alpha zein with two subgroups-19KDa and 22KDa, contributes >60% of total zein proteins in the maize endosperm along with other zein proteins (beta-15KDa, gamma-50, 27, 16KDa, and delta-10KDa) [12, 13]. The zein fraction in normal maize contains higher proportion of leucine (18.7%), phenylalanine (5.2%), isoleucine (3.8), valine (3.6%) and tyrosine (3.5%) and relatively small amounts of other essential amino acids such as threonine (3%), histidine and cysteine (1%), methionine (0.9%), lysine (0.1%). It is devoid of another essential amino acid tryptophan, which is absent from the maize alpha–zein portion. While the non-zein fraction is well-balanced and high in tryptophan and lysine [14]. The problems of deficiency have been mainly dealt with by supplementing nutritionally improved grains with lysine and tryptophan by bacterial fermentation. However, this approach is very expensive, and amino acids are often lost in processed food. Hence, it is critical to use a genetic enhancement technique in which necessary amino acids are enhanced (or) integrated into grain proteins [15].

There are continuous efforts and investments including a lot of time and effort to enhance the quality of the protein to rectify these nutritional deficiencies. After much effort, in the 1920s, a mutant known as an *opaque-2* (*o2*) was identified in Connecticut, USA [16]. However, it wasn't until 1961 that scientists realized that homozygous recessive *o2* alleles, which cause more lysine and tryptophan to accumulate in the endosperm of maize grain compared to non-mutant zein, provide a nutritional advantage. A considerable increase in lysine (69%) was found in maize endosperm when a homozygous combination of recessive *o2* alleles was analyzed at Purdue University in the United States [17].

Discovery of lysine-rich mutant genes

Several mutant genes useful for lysine enhancement and nutrient-rich maize have been discovered by different researchers to overcome challenges and limitations faced by the additional effects of previous mutant genes. Here are some of the specific mutant genes related to the enhancement of lysine in maize listed in Table 2.

Genetic mechanism of high lysine mutant alleles

A random mutation in nature *opaque-2* was created in which Lysine and tryptophan levels are increased by a factor of two. Mechanism explained in Figure 1. The transcription factor basic - leucine-zinc domain is broken due to a mutation in the *o2* gene. The 22-kilodalton alpha-zein [18] and

Table 2. Different mutant genes useful for lysine improvement in maize

Gene	Symbol	Year	Chromosome number	Reference
opaque2	*o2*	1964	7	[1]
floury2	*fl2*	1965	4	[2]
opaque7	*o7*	1972	10	[3]
opaque6	*o6*	1975	8	[4]
floury3	*fl3*	1975	8	[4]
mucronate	*Mc*	1983	2	[5]
Defective endosperm	*De-B30*	1997	7	[5]
opaque16	*o16*	2005	8	[6]

may be additional lysine ketoglutarate reductase enzyme genes are controlled by this protein (LKR). Thus raising the non-zein percentage, the mutant allele *o2* dramatically raises the lysine content [24, 25].

Free lysine levels rise due to the faulty LKR enzyme (Figure 2). The *floury2* mutant allele genes for a 22-KDa alpha-zein that lacks a functional signal polypeptide, which decreases the zein fraction and raises the non-zein fraction. Transgenic production of the gene for the alpha-zein subunit 19-KDa protein with an S15P mutation in the signal peptide was sufficient to generate opacity in the *de-B30* mutant. The *Mc* gene, in contrast to the preceding two instances of signal peptide mutations, encodes a 16-KDa gamma-zein with a frameshift mutation. Recessive mutations include those for opaque (*o1, o2, o5, o9,11, o13, o16,* and *o17*), floury (*fl1, fl2,* and *fl3*), and *mucronate (muc)* and *defective endosperms (de)*. Opaque mutations changed the regulatory network [9, 17, 19], whereas floury, mucronate, and faulty endosperm mutants altered the amino acid storage protein profile [10]. Translucent16, another mutant allele with a similar impact as *o2*, also observed elevated levels of lysine and tryptophan in the endosperm.

Problems challenges while using lysine mutant genes

While developing QPM with these mutant alleles, researchers had to deal with a wide variety

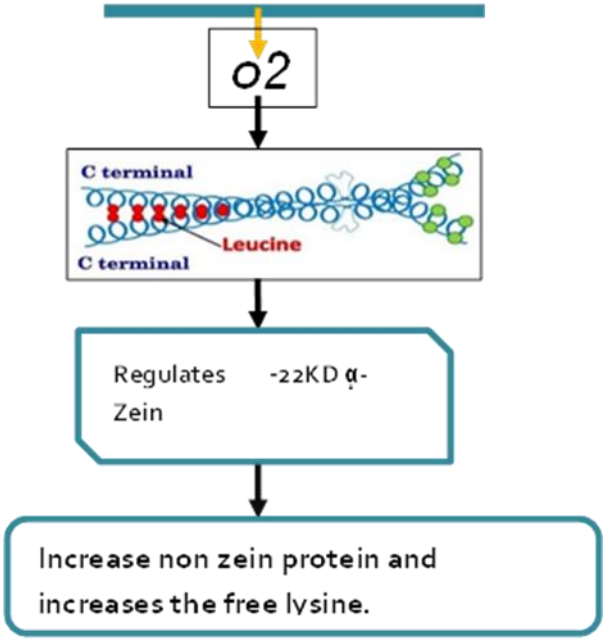

Figure 2. The mechanism explaining the opaque-2 mutation in maize

of unwanted pleiotropic consequences. For instance, soft endosperm, which negatively influenced dry matter buildup, made plants more vulnerable to pests and diseases, decreased kernel density, and ultimately reduced production. When a kernel's endosperm is too soft, it might be damaged during post-harvest storage (Figure 3). These lines are rejected by farmers for cultivation because of their unintended side effects and reduced maize production may be attributed to the lack of interest in QPM lines among farmers [26].

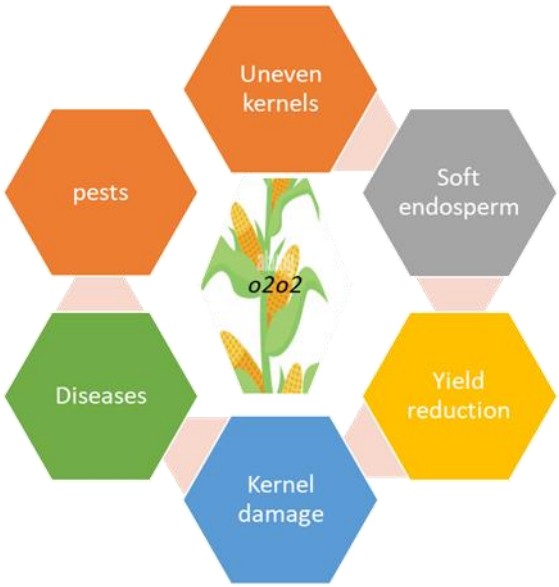

Figure 3. Problems caused while using opaque-2 mutant gene

Researchers again have to design specific strategies and start their work to overcome these negative effects. They identified two approaches to overcome these problems so that QPM cultivars can attain acceptance among various clients.

1. Using double mutant genes.
2. Simultaneous use of recessive homozygous opaqe2 gene along with modifier genes.

As per elaborated analysis o2 and other mutations related to endosperm quality have been shown to form non-vitreous double mutant combinations [14]. It is to be noted that, the best strategy so far has been to use genes that modify endosperm and amino acids [27].

Genetic systems of QPM

Genetic systems to overcome pleiotropic effects [9, 26] are listed below.

1. The recessive *o2* mutant gene
2. Endosperm modifier genes
3. Amino acids modifiers genes

Useful modifier genes successfully suppress the starchy *o2* phenotype with little to negligible impacts on protein quality, and various opaque mutant genes are helpful for supporting QPM [28]. Since these modifier genes are inherited at several loci [29], studying the genetic basis of QPM as well as using them in specific strategies is a complex endeavor.

The recessive homozygous o2 gene

Expression of the ribosomal inactivating protein-encoding gene and the alpha-zein coding (22-KDa) gene is inhibited by a mutant allele of the opaque-2 gene, which results in a faulty basic domain of the leucine-zipper transcription factor [30]. Mutations in the opaque-2 gene cause alpha-zein to shrink to

about one-tenth of its normal size, thereby altering the packing of starch in the grains during maturity (or desiccation) and making the grains mushy. Inversely, when the alpha-zein fraction decreases, the gamma-zein [31] proportion grows and the kernel becomes more rigid due to the gamma-zein concentration. The synthesis of non-zein proteins, such as lysine and tryptophan levels, is increased when homozygous recessive *o2* alleles are present [10]. The opaque-2 mutation causes a decrease in the activity of the enzyme that breaks down free lysine, resulting in a toxic waste product and defective enzymes lead to an increase in the endosperm's free lysine concentration [25].

Endosperm modifier genes

The second genetic mechanism for QPM is the endosperm modifier gene system that makes the endosperm of maize kernels more rigid without compromising the grain's protein content. As gamma-zein levels correlate with endosperm hardness, modifying genes enhances translation by interacting with the gamma-zein mRNA transcript. An increase in protein bodies and vitreous grain formation has been linked to overexpression of gamma-zein. Selecting for these en-modifiers and the o2 mutant allele can be done quickly and cost-effectively by using light meters. whereby transparent granules transmit light while opaque grains absorb it. The degree to which the endosperm of grain is opaque may range from 1 (totally hard/vitreous) to 5 (soft/opaque) [32]. Here are how the rankings shake out: Not opaque (Type 1), somewhat opaque (25% opaque) To illustrate, intermediate scales contain different degrees of opaqueness, and, Type 3 is 50% opaque while Type 4 is 75% opaque. Opacity level 5 is complete darkness as reported by Dev et al., [33]. All grains with a score between 2 and 5 are homozygous for the o2 allele, but only grains with a score of 2 or 3 have significantly changed hard endosperm modify the norms or good lines and are thus eligible for selection as QPM grains. Two genetic loci responsible for endosperm hardness modification in *o2o2* backgrounds have been mapped to the long arm of chromosome 7 using this semiquantitative method [32], and one endosperm modifier locus maps curiously close to a gamma zein gene, gzr1 (Maize Genome Project www.maizegdb.org).

Amino acid modifier genes

Lysine and tryptophan are also influenced by amino acid modifier genes. These changes are influenced by modifier genes and vary from generation to generation [34]. Depending on the genetic background, the lysine content of normal maize may range from 1.6% to 2.6%, while the lysine content of QPM maize can range from 2.7% to 4.56%. However, the lysine content of whole grain flour for both types of maize is typically around 2% and 4%, respectively. It has been shown by Hernandez and Bates [35] that lysine and tryptophan levels are positively correlated, implying that a quantitative test for either amino acid may be used to assess protein quality. Tryptophan is used more often than it should be because of its lower price relative to other laboratory supplies. Many genes have been identified as playing a role in controlling protein building blocks called amino acids. Three separate gene loci were identified on chromosomes 2, 4, and 7 that were discovered to influence protein synthesis and, more specifically, lysine concentration [36]. In the same genetic mapping studies, researchers measured the quantity of free amino acids (including lysine) using a separate ninhydrin test and uncovered nine significant loci on chromosomes 1, 2, 3, 4, 5, 7, 8, and 9. These studies demonstrate that the formerly simple genetic make-up of opaque-2 maize has transformed into a conventional polygenic trait about QPM, necessitating its treatment as such in breeding programs. Even if the *o2o2* genotype is maintained, further protein quality increases may be lost if lysine and tryptophan levels are not routinely checked throughout breeding [37]. These genetic systems may be used in conjunction with different breeding strategies to advance QPM.

Breeding strategies for QPM

New QPM cultivars are developed mainly by the two approaches, such as conventional and non-conventional breeding strategies.

Conventional breeding method

The primary objective of QPM breeding is to create agronomically superior maize with a high yield that is also abundant in all of the necessary amino acids. Due to the high concentration and high quality of germ protein, it was originally the goal of breeding to alter the proportion of germ to endosperm. When the germ/endosperm ratio rises, so does the quality and amount of the protein in the seed [38]. However, this strategy fails due to consequences of higher germ, because larger germs are difficult to store after harvest, shortening their useful life. Next, the available genetic variability was explained to increase lysine content by the strategy of recurrent selection. However, this was equally ineffectual on account of so little genetic variability in lysine content to begin with. Using a cutting-edge technique called "Modified Back Cross cum recurrent selection," it was possible to rapidly transform non-QPM lines into QPM ones [14, 28]. World Food Prize-2000 was confessed to Surinder K. Vasal (India) and Evangelina Villegas (CIMMYT) for their comprehensive work on the creation of QPM. Heterosis breeding is the primary method used to boost output and productivity because of the proven benefits of technology making higher yields possible with hybrids. First, inbred lines and donor genes must be developed so that hybrid combinations are made and potential combinations may be created. These inbred lines need to be created by breeding individuals within the same population rather than using donor stock. These offspring are then bred back to the recurrent parent until the whole recurrent parent genome, including any donor genes, can be retrieved. Each successive generation has been selected for lines that share characteristics with repeating parents and donor genes. It is possible to create QPM hybrids by crossing inbred lines that have been tested and shown to have an abundance of lysine and tryptophan. Typically, it will take around 7–8 generations using normal breeding strategies and techniques to produce a new variety. The generation period for developing hybrids can be cut down to only three or four generations by using the molecular breeding approach.

Molecular breeding strategies

To overcome the drawbacks of conventional breeding methods, this strategy makes use of a wide variety of molecular markers. The recessive nature of the opaque-2 gene and the polygenic inheritance of modifier genes contributes to the requirement of a long time to convert normal maize lines to QPM lines [2]:

1. To find the homozygous recessive after each backcross, selfing is necessary since recessive alleles are inherited as a single copy. It takes at least six backcrosses to retrieve the whole genome of the repetitive parent.
2. Selection of modifier genes along with homozygous recessive opaque-2 gene.
3. Conventional breeding requires more labor, time, and funds for biochemical analysis of lysine and tryptophan content.

The challenges were addressed by using several genetic markers in a molecular breeding strategy. As a result of MAB's efforts, genetic progress was hastened, selection efficiency was increased, and high-yield varieties were created [2, 34]. Molecular-assisted backcross breeding was used with foreground and background selections, by using three markers (*phi057, umc1066,* and *phi112*) within the opaque-2 gene on the chromosome 7 short arm as illustrated in Figure 4 [39-41]. As a dominant marker, *phi112* is incapable of distinguishing between homozygotes and heterozygotes. On the other hand, homozygotes and heterozygotes may be distinguished thanks to the codominance nature of *phi052* and *umc1066* [42-44]. By using these markers, donor alleles can be recognized at early generations with foreground selection in heterozygotes plants, and these

background selections identify and select instances of parental genome recovery [2]. By using marker-assisted backcross breeding (MABB), inbred lines can be created in as few as three to four generations, with >99 percent of the parental genome and genes of interest being recovered. After this step, inbred lines are subjected to lightbox screening to sort kernels by opaqueness, and such a stepwise strategy helps to determine which ones should be selected and cultivated.

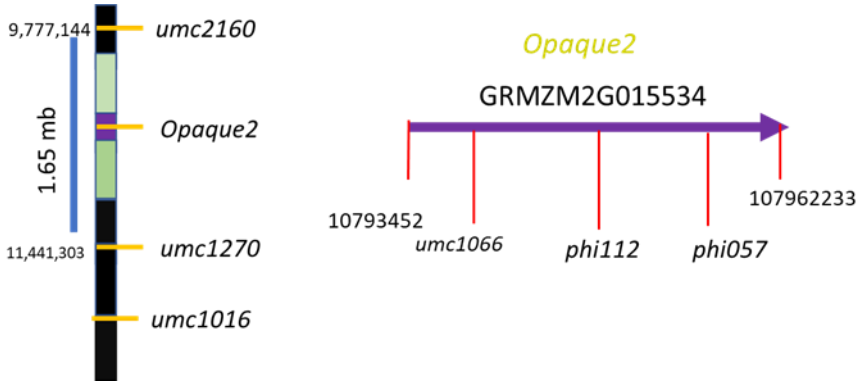

Figure 4. Physical position of the opaque-2 gene on chromosome 7

Lysine and tryptophan levels were determined using biochemical analysis. By using VQL1 and VQL2 as parental lines Vivek QPM-9 hybrids were developed [45, 46]. These parental lines when compared to the normal form of the Vivek-9 hybrid, have increased nutritional content (lysine by 30%, tryptophan by 41%, histidine by 23%, and methionine by 3.4%), but decreased leucine by 12%. Lines were developed from CM212 X CML180 and CM145 X CML170 crosses. CML180 and CML170 are donor stocks. Foreground and background selections were used for identifying genes of interest and recovered recurrent parent genomes in the inbred lines. Those lines which are similar to the recurrent parent and nutritionally high lysine and tryptophan are used as parental lines for the development of the Vivek QPM-9 hybrid. It has to increase the nutritional content (lysine by 30%, tryptophan by 41%, histidine by 23%, and methionine by 3.4%) as compared to the normal version of the Vivek-9 hybrid, whereas leucine was decreased by 12%. Some of the released QPM varieties developed in different countries are listed in Table 3. Prasanna et al., [15] reported, that the QPM version of elite inbred lines was used to develop hybrids. QPM versions of these hybrids were 'Pusa HM4 improved', 'Pusa HM8 improved', and 'Pusa HM9 improved', which were released for commercial cultivation in 2017 in India [47].

Table 3. List of QPM cultivars released in different countries

Cultivars	Variety type	Year of release	Country
Shakti	OPV	1970	India
Rattan	OPV	1970	
Protina	OPV	1970	
Shakti 1	OPV	1997	
Shaktiman 1	Hybrid	2001	
Shaktiman 2	Hybrid	2004	
HQPM 1	Hybrid	2005	
Shaktiman 3	Hybrid	2006	
Shaktiman 4	Hybrid	2006	
HQPM 5	Hybrid	2007	
HQPM 7	Hybrid	2008	

Vivek QPM 9	Hybrid	2008	
Pusa HM4	Hybrid	2017	
Pusa HM8	Hybrid	2017	
Pusa HM9	Hybrid	2017	
Dadaba,	Hybrid	1977	
Mamaba	Hybrid	1977	Ghana
CIDA-ba	Hybrid	1977	
BHQP542	Hybrid	2001	
Melkasa6Q	OPV	2008	
BHQPY545	Hybrid	2008	Ethiopia
AMH760Q	Hybrid	2011	
MHQ138	Hybrid	2012	
Melkasa1Q	OPV	2013	
BHQPY548	Hybrid	2015	
BR 451	Hybrid	1988	Brazil
BR 473	Hybrid	1994	
Zhongdan 9407	Hybrid	1999	China
WSQ104	OPV	2000	
KH500Q	Hybrid	2003	Kenya
KH631Q	Hybrid	2003	
Kh531Q	Hybrid	2004	
LISHE H1	Hybrid	2003	
LISHE H2	Hybrid	20033	
TANH611	Hybrid	2006	Tanzania
NATAH6Q	OPV	2013	
MAMS H0913	Hybrid	2014	
Longe-5	OPV	2000	
NALONGO	OPV	2002	
SPALONGO	Hybrid	2008	Uganda
VP Max	OPV	2012	
Longe-5	OPV		South Sudan
Sussuma	OPV	2003	Mozambique
OLIPA	Hybrid	2008	
Chitedze 2	OPV	2008	Malawi
MH29	Hybrid	2009	
QS7608	Hybrid	1996	
QS7701	Hybrid	1996	
QS7606	Hybrid	1997	
QS7705	Hybrid	1997	
HL-1	Hybrid	2000	
HL-2	Hybrid	2000	South Africa
HL-8	Hybrid	2000	
QS7703	Hybrid	2001	
QS7707	Hybrid	2002	
QS7709	Hybrid	2002	
QS7614	Hybrid	2003	

QS7616	Hybrid	2003	
QS7646	Hybrid	-	
QS7715	Hybrid	2004	
QS7751	Hybrid	2005	
QS7761	Hybrid	2006	
QS7717	Hybrid	2006	
QSOba	OPV	2008	
Obatanpa SR	OPV	2009	
Qs-king	OPV	2010	
QS-Mini	OPV	2012	
Nelsons choice QPM	OPV	2012	
CAP9006QS	Hybrid	20013	
CAP9444NG	Hybrid	2014	
CAP9015	Hybrid	2014	
SA41115Q	Hybrid	2015	
JEMAT601Q	Hybrid	2015	
Obatanpa	OPV	2004	Zambia
GV682P	Hybrid	2015	
GV687P	Hybrid	2015	
Poshilo Makai-1, (white kernel)	Hybrid	2008	Nepal
Poshilo Makai-2 (yellow kernel)	Hybrid	2017	
QPMH200	Hybrid	2017	Pakistan
QPMH300	Hybrid	2017	

opaque 16 is a second recessive mutant discovered by Yang et al., [48]. Robertson's Mutator stock yielded the mutant *o16.* Two simple sequence repeat (SSR) markers, *umc 1141* and *umc 1149*, were located on chromosome 8 and shown to be associated with the o16 mutant. It was also observed by Yang et al., [23] that a mixture of *o2o2* and *o16o16* had 30% more lysine than either component alone.

Higher lysine levels (0.616 percent in flour) were reported in *o2* and *o16* mutants introgressed into the waxy background by Zhang et al., [49], in comparison to *o2o2* segregants (0.555 percent in flour). Two Chinese waxy lines (QCL 5019 and QCL 5008) were pyramided with the o16 allele by Yang et al., [50] using MAS. These inbred lines, which are derived from the *o16o16* population, have 16-27 percent and 18-28 percent greater lysine levels than waxy parents, respectively.

In a similar vein, maize genotypes with fewer anti-nutritional components have been developed via MABB [51]. Marker-assisted introgression of the ß-carotene hydroxylase allele allowed Muthusamy et al., [52] to create ß-carotene-rich maize hybrids. A key locus (y1) for carotenoid concentration was discovered by Chander et al., [53] utilizing a gene-targeted molecular marker (*Y1ssr*).

Another approach to increase lysine is RNAi. A greater amount of transgenic lysin is produced by silencing of endosperm-specific lysine metabolism [54] or accumulation of alpha-zeins with RNAi. The latter method expresses similar to the *o2* phenotype. RNAi causes knockdown of 22-KDa [55] and 19-KDa [29, 56] alpha-zein. These transgenics increase the lysine by 15-20%, which shows that the transgenic approach is useful to increase lysine. The second major finding of these studies was that the reduction of alpha-zein protein synthesis was sufficient to induce an opaque endosperm

phenotype. 22-KDa RNA interference lines show more interaction with beta and gamma-zein proteins [28] than 19KDa. The absence of 22-KDa protein leads to the permission of 19-KDa to the center of the protein body. It disrupts the protein body morphology and its interaction with beta and gamma-zein. It leads to an opaque endosperm phenotype. To create T1 seeds for genetic research, T0 transgenic pollen was used to fertilize the hybrid of B73 and Mo17. Zein proteins were isolated from six normal and "green" immature kernels. The six green kernels' levels of the 22- and 19-kDa "-zeins" were significantly lower than those of the six regular kernels. At maturity, there was a 1:1 segregation between the vitreous and nongreen kernels (216), and the opaque and green kernels (222) [57].

Seed DNA-based genotyping for QPM

Obtaining leaf samples for DNA genotyping throughout the germination process requires careful labeling and tracking. Seed DNA genotyping for marker-assisted QPM breeding has been established in recent years at CIMMYT. These techniques generate both high-quality and quantity DNA from a wide variety of maize seeds. One of the main benefits of this approach is that it allows for genotype selection before planting, which, by limiting the number of seeds distributed to just those that have the recessive *o2* gene, may greatly improve the success rate of non-QPM X QPM crosses. Light-based endosperm hardness screening is simplified and facilitated by this technology, which also minimizes labor expenses, time, and score errors. The cumulative and rapid increases in selection pressure across several breeding cycles predicted by Babu and Prasanna [34] seem likely under these circumstances.

Conclusion

Maize is a staple crop in developing countries, whose endosperm is deficient in lysine and tryptophan. The deficiency of these essential amino acids makes normal maize incomplete food and causes severe diseases like Kwashiorkor, while its consumption reduces the niacin impact. After the discovery of the mutant *opaque2* gene, nutritional content gets doubled enhancing nutrient quality. Several other mutant genes were also discovered. However, these mutant genes showed pleiotropic effects, and due to these negative effects, these QPM varieties are not popular. Then modifier genes along with mutant *o2* gene with modified back cross cum recurrent selection were used. Conventional breeding required 6-7 generations and also selfing after each backcross to get recessive homozygous *o2* alleles was not efficient and required more labor. To overcome these, the MAS strategy was used with *phi112, phi057,* and *umc1056* markers located within the *o2* gene. These markers are useful to identify the *o2* gene at an early generation and to facilitate recovery and a more efficient approach for obtaining recurrent parent genomes. As a practical utility, Vivek QPM hybrid-9 was developed by using MABB.

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