Cereal Research Communications (2022) 50:559–572
https://doi.org/10.1007/s42976-021-00211-8

REVIEW

Nutritional benefit and development of quality protein maize (QPM) in Ethiopia: review article

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Received: 8 July 2021 / Accepted: 9 September 2021 / Published online: 5 October 2021

Abstract

Among the top three cereal crops producing in Sub-Saharan Africa (SSA), Asia, and Latin America, maize (Zea mays L.) is a major source of food security and economic development for these countries. However, maize lacks a required amount of the essential amino acids lysine and tryptophan in its endosperm. In the mid-1960 breeding for improved protein quality in maize was started to overcome the two amino acids deficient in maize endosperm proteins, and came up with the discovery of mutants, such as opaque-2, that produce enhanced levels of lysine and tryptophan. Despite these achievements, adverse pleiotropic effects imposed severe constraints on the successful exploitation of these mutants. A collaboration work from different background disciplines of researchers corrected the negative features of the opaque phenotypes has ensured the rebirth of QPM after long efforts. QPM has twice the amount of lysine and tryptophan, as well as protein bioavailability that rivals milk casein when comparing with conventional maize types. It was confirmed that 100 g QPM is required for children and nearly 500 g for adults to maintain the adequacy of lysine and tryptophan. Relatively this represents a 40% reduction in maize intake to common maize to meet protein requirements. Therefore, this review will highlight the history of developing QPM, the efforts that have been made to recovery technical problems raised to develop nutritionally enriched maize successfully through both conventional and molecular breeding methods, and indicating the need for intensification of efforts to create a greater impact on malnutrition in maize consuming populations, especially in developing countries.

Keywords Bio-fortification · Food security · Low income country · QPM

Introduction

The major challenge in a developing country is the food security situation. Out of 0.8 billion people undernourished in the world around 28% are living in sub-Saharan Africa and of which more than half are living in East Africa (Semahegn 2021). Regarding the current food situation in Ethiopia, about 25 million people have faced a high level of acute food insecurity (IPC 2021).

Globally, maize is becoming increasingly important as a staple food for the poor people so as it is the cheapest cereal (Akalu, Taffesse, Nilupa S Gunaratna, et al. 2010b). Following rice and wheat, currently, maize stands as the third important crop in the world since it gained growing popularity as a food and feed crop (Penning et al. 2009). In America Maize and its products constituted 30% of the food supply, in Africa 38%, and 6.5% in Asia, and thus, is a major source of food security and economic development in Sub-Saharan Africa (SSA). Maize is a major staple food and the most important energy source (CSA, 2018), with intakes ranging from 50 to > 330 g/person/day, and providing daily energy, protein, and micronutrients (Prasanna et al. 2020). In Ethiopia, millions of people depend on maize as a staple food crop. It is currently grown by 9 million households in 2 million hectares of land from which more than 7.0 million metric tons are produced annually. It contributes about 29% of the calorie intake from total cereal consumption, followed by wheat and teff which contribute about 21 and 17%, respectively (CSA 2017).

Since the early 1900s significant efforts have been made to develop well-adapted and high-yielding hybrid and open-pollinated varieties for different agro-ecologies (Abate et al. 2015). These improvement results, along with applying improved agronomic practices helped farmers to produce a mean grain yield of 6 t/ha. As a result maize production

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and productivity grew much from 1.5 million tones and 1.7 t/ha within the early 1900s to their current level of seven million tones and 3.7 t/ha, respectively (Jilo and Tulu 2019). An increase in production of this staple crop is believed to invariably cause higher consumption and reduced poverty across households in Ethiopia. However, malnutrition has been found as a chronic problem and worsening in Sub-Saharan Africa (James 2010).

Though the production and productivity of maize are highest comparing with other cereal crops in Ethiopia, maize varieties presently grown by farmers, hereafter referred to as conventional maize (CM) varieties, are deficient in two essential amino acids, lysine and tryptophan (Ranum, Peña-Rosas and Garcia-Casal, 2014; Akalu, et al. 2010b). Monogastric animals including human beings enabled to synthesis such essential amino acids through the metabolic process unless they consumed the food fortified with these essential amino acids. A particular problem among young children and pregnant and/or lactating women whose diet is dominated by maize and who have limited alternative sources of these amino acids have been suffered from protein deficiency due to the failure to obtain essential amino acid from their daily diet. An important factor that determines protein quality is how closely the ratio of essential amino acids present during a particular food item matches the human requirement. Animal products like meat, eggs, and milk, and legumes crop are not affordable for a large segment of small-scale farmers though these products are known to be a good source of essential amino acids (Akalu et al. 2010a). To achieve a balanced amino acid pattern, a maize-based diet must have sufficient quantities of legumes or animal-source foods. However, these foods are often not available or are prohibitively expensive (Pellett and Ghosh 2004; Bouis 1999). On the other side, beans are the major legume in Africa (Akalu et al. 2010a), but their long cooking time is a concern because of the decreased availability of wood and charcoal for fuel and the increased price of alternative fuels. To overcome this problem, researchers have used conventional breeding methods to develop maize cultivars that have higher lysine and tryptophan content than CM genotypes and a vitreous endosperm like that of CM to ensure acceptable kernel characteristics. When modified to supply a vitreous endosperm resembling that of CM, maize that contains approximately double the quantity of lysine and tryptophan has been named as "quality protein maize" (QPM) which is a cheap source of protein, given that farmers can grow, manage, harvest, and consume it in the same way they do CM varieties (Wegary et al. 2015).

Since it is developing the QPM has been extensively studied toward it is a nutritional advantage worldwide by different researchers. The study conducted in the clinical setting in Colombia, Guatemala, and Peru compared conventional maize to a greater proportion of protein from o2 maize dieter indicated that the children were recovered from severe malnutrition that the o2 is also available for utilization by the body for normal physiological process and growth (Krivanek et al. 2007). A report driven from an experiment conducted in the controlled environment in Peru at Clinical work involving children recovering from severe malnutrition indicated that the children consuming an entirely QPM-based diet had a similar growth rate to the same children consuming a modified cow's formula (Akalu et al. 2010b). A 6-month study that provided a daily meal made of o2 maize, conventional maize, or skims milk to preschool children from low-income families in New Delhi, India, found that children consuming o2 maize had weight, height, chest, and mid-upper-arm measurements like or only slightly less than those of youngsters consuming skimmed milk and greater than those of youngsters consuming conventional maize (Toro et al. 2003).

Children who consumed QPM had better growth in terms of height, weight, height-forage particular in Southern Ethiopia where the population relies heavily on maize (Tuberosa, Graner and Frison, 2014; Knoll et al. 2008), and weight-forage but QPM consumption did not have an effect on diarrheal episodes of respiratory infection as a report of a 3.5-month study that provided a daily snack made of QPM or conventional maize to mild moderately malnourished 1-to 5-year olds in a Nicaraguan day center (Ignjatović-Mičić et al. 2010). The effect of feeding infants a traditional maize porridge made from either QPM or conventional maize found that infants consuming the QPM porridge had significantly fewer sick days and less stunting than children consuming conventional maize. As a study report in the Ashanti Region of Ghana indicated and a similar follow-up study in the same region found greater weight gain among infants consuming QPM (Akalu et al. 2010b). (Akalu, Taffesse, Nilupa S Gunaratna, 2010b) reported that in Ethiopia, by great in Sibu Sire Woreda and East Wollega where maize is a dominant crop, demonstrated the positive effect of QPM on both the peak and weight of youngsters aged 7 to 56 months.

The information obtained from a focus group discussion in Sibu Sire Woreda confirmed that traditional foods prepared with QPM were appreciated by the farmers for their taste and cooking qualities. Designed experimental studies in eastern African countries also indicated that QPM is more acceptable and even preferred over CM for preparing widely consumed food products such as *ugali* in Tanzania, G*itheri* in Kenya, and *injera* in Ethiopia. Therefore, this review is designed to show the historical development, the nutritional benefits, Genetic implication background, and current status of QPM in Ethiopia and at global aspects.

Breeding efforts versus History of QPM development

QPM development dates back to the 1920s when a natural spontaneous mutation of maize with soft and opaque grains was discovered in a maize field in Connecticut, USA (Vivek et al. 2008). The salient events of this discovery (Li and Vasal, 2015a) are summarized as follows: Kernels of the mutant maize were delivered to the Connecticut Experiment Station and the mutant was eventually named *opaque2* (*o2*) but received little further attention. In 1961, researchers at Purdue University, USA, discovered that maize homozygous for the *opaque2* (*o2o2*) recessive mutant allele had substantially higher levels of lysine and tryptophan in the endosperm, compared to CM with the dominant *O2* allele (*O2O2* or *O2o2*) (Tuberosa, Graner and Frison, 2014; Schmidt et al. 1990). Further experimentation in the 1980s demonstrated that the increased tryptophan content in *o2* maize effectively doubled the biological value of the maize protein, thus reducing by half the amount of maize that needs to be consumed to get the same amount of biologically usable protein in a maize diet.

At a worldwide level breeding programs started converting conventional maize to *o2* versions through a direct backcross approach. However, serious negative secondary (pleiotropic) effects of the mutation were soon discovered which severely limited the practical use of the mutation in the field. Musila et al.(2010) investigated these negative effects as fellows: yield loss of up to 25% due to the lower density of the soft endosperm of *o2* grains, as well as increased susceptibility to fungal ear rots and storage pests; and unacceptability of the soft endosperm texture to consumers who are accustomed to harder grain types. The pleiotropic effects, especially the low yield and soft kernels of the *opaque2* mutation, restricted the usefulness of this mutation in breeding programs. However, screening of hard kernels in some of the backcross-derived populations at CIMMYT paved the way for developing *opaque2* varieties with hard kernels (Li and Vasal, 2015b). In response to these limitations CIMMYT's QPM breeding efforts focused on: converting a range of subtropical and tropical lowland adapted conventional maize populations to *o2* versions through backcross recurrent selection, regaining the original hard endosperm phenotype of the converted populations/lines; and maintaining protein quality while increasing yield and resistance to ear rot.

After a long effort, CIMMYT's breeding program came up with maize genotypes developed was termed Quality Protein Maize (QPM) which is characterized by having higher lysine and tryptophan content than CM, also as normal vitreous endosperm, reduced susceptibility to post-harvest insect pests, and diseases like ear rots, as compared to their o2 predecessors, and its yield is comparable to or higher than that of CM grown by farmers ("AGRICULTURE & FOOD May 2020," 2020).

QPM looks and performs like conventional maize and can be reliably differentiated only through laboratory tests. Furthermore, a number of scholars tended to realize the genetic differences between the CM and QPM through different scientific procedures. Several QPM populations and pools possessing different ecological adaptation, maturity, grain color, and texture were developed (Li and Vasal, 2015a). A number of advanced maize populations in CIMMYT's Maize Program were successfully converted to QPM populations. QPM development took over three decades of painstaking research; two CIMMYT scientists, maize breeder Surinder K. Vasal and cereal chemist Evangelina Villegas received the 2000 World Food Prize for their significant contributions to QPM development (Babu et al. 2005).

Currently, CIMMYT has extended strong breeding strategies to QPM focus on pedigree breeding wherein the best performing inbred lines with complementary traits are crossed to establish new segregating families. Both QPM × QPM and QPM × non-QPM crosses are made depending upon the specific requirements of the breeding project (Ignjatović-Micić et al. 2010). In addition, backcross conversion is used to develop QPM versions of parental lines of popular hybrid cultivars that are widely grown in CIMMYT's target regions. Ignjatović-Micić et al. (2010) experimented to modify a kernel tryptophan content on F3 and BC1F1 generations of QPM x opaque2, opaque2 x QPM, and standard lines x QPM crosses. They reported that a whole grain tryptophan content in F3 and BC1F1genotypes of crosses between QPM and opaque2 germplasm was at the quality protein level, with few exceptions, and all BC1F1 genotypes of standard lines x QPM had tryptophan content in the range of normal maize while the majority of F3 genotypes had tryptophan at the level of QPM Fig. 1. With regards to molecular marker-assisted selection (MAS) for generating QPM versions of elite inbred lines, significant strides have also been made. Marker-assisted selection (MAS) has been used to accelerate the pace of the QPM conversion program for that, the microsatellite markers located within the o2 gene. In 2008, Marker-assisted backcross breeding (MABB) for o2 led to the development and release of a single-cross QPM hybrid, 'Vivek QPM-9' was initiated in India (Gupta et al. 2013). Compering with the original hybrid (Vivek Hybrid-9), 41% tryptophan and 30% of lysine content has been observed in 'Vivek QPM-9'. Using Marker-assisted backcrossing in different genetic backgrounds, the Institute of Crop Science, Chinese Academy of Agricultural Sciences (CAAS) was developed a diverse QPM inbred lines (Rajendran et al. 2014; Parajuli et al. 2020). An enhancement of 23% lysine in o2o2/o16o16 progeny over the o2o2 inbred comparison from a combination of o2 and o6 has been reported (Zhang et al. 2012). Recent technological

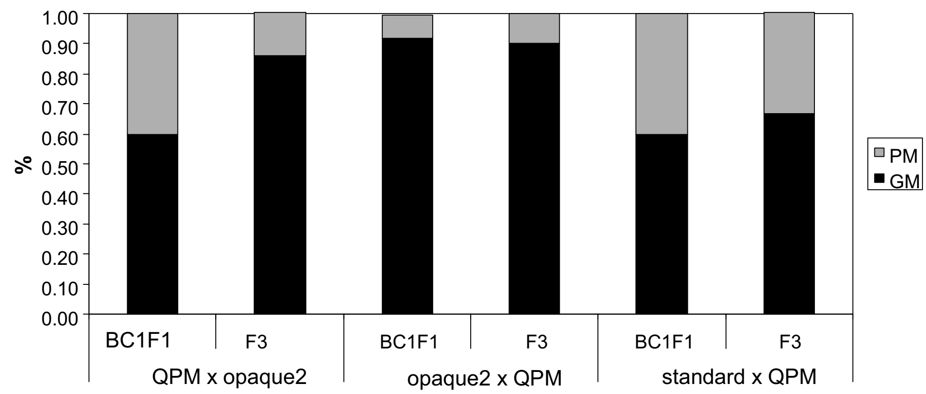

Fig. 1 Kernel modifications in different type of crosses and generations Legend: **PM**–poor modifications kernel types, and **GM**–good modifications kernel types. Source: adapted from Ignjatović-Micić et al. (2010)

developments, including high-throughput, single seed-based DNA extraction, coupled with low cost, high-density SNP genotyping strategies and breeder-ready markers for some key adaptive traits in maize, promise to enhance the efficiency and cost-effectiveness of MAS in QPM breeding programs is undertaking (Babu et al. 2013).

In order to develop QPM hybrids with enhanced levels of lysine and tryptophan, Marker-assisted breeding has also been initiated in India to combine both o2 and o16 (Sarika et al. 2018; Prasanna et al. 2020). Using MABB, four introgressed of o16 commercial parental lines of QPM hybrids (HQPM-1, HQPM-4, HQPM-5, and HQPM-7) were released in India (Sarika et al. 2018). An average enhancement of 49 and 60% in lysine and tryptophan over the original hybrids, with the highest enhancement amounting to 64 and 86% of reconstituted hybrids have been observed respectively.

Compared with conventional Maize (CM), opaque-2 yielded less grain, and its grain weighed less, had higher moisture at harvest, and succumbed more to fungal infections and storage insect infestations were the main challenge that arose in early 1970 at the time of QPM technology was commenced. Many users disliked the grain's dull and chalky appearance, having been accustomed to hard and glossy kernels Fig. 2. By the mid-1970 interest had declined by processors almost to the vanishing point due to the reason the flour texture of soft kernels, which were more difficult to store and to mill (Ignjatović-Micić et al. 2010).

The limitations brought a turning point in breeding efforts, in order to modify soft o2 developed materials. Among a lot of records, a notable effort was that of a small team of maize breeders at the Centro Internacional de Mejoramiento de Maiz y Trigo (CIMMYT), and at the University of the Natal, South Africa started to carefully examine the nature and seriousness of inherent problems and came out with viable strategies to overcome the problems (Bantte and Prasanna, 2003). In Mexico, they continued improving the agronomic qualities of the maize for 10 years in their laboratories and fields. They claimed to have developed experimental varieties with high nutritive quality, high

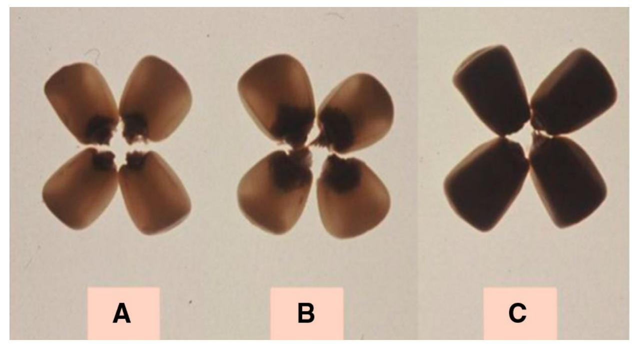

Fig. 2 Fig. 3. Back-lit maize kernels illustrating the phenotypic differences of o2 mutation. **a** Common maize; **b** QPM; **c** o2 maize without modification **Source:** Nuss and Tanumihardjo, (2011)

yields, normal moisture content, traditional appearance, and conventional hardness in early 1980. This led to the recognition of the 'o2 endosperm modifier gene' that alters the phenotype of o2 mutants, giving them a normal hard (vitreous) appearance instead of a soft, chalky nature. By 1986, they had, it seemed, fundamentally transformed opaque-2 maize into maize that was 'normal' in all respects except for its superior nutritional value. They called the new variety 'quality protein maize (QPM). A 50% translucent and 50% opaque kernel endosperm modification was reported by (Li and Vasal, 2015a) for the first time. Then after a number of works studied modified o2 kernels and observed a varying proportion of translucent and opaque fraction (Wessel-Beaver and Lambert, 1982; Sevanayak and Gupta, 2018) Fig. 3. Due to their complex nature of inheritance, the strategy of selection for endosperm modification in o2 background has been highly effective in ameliorating the negative features of the opaque phenotype, since these genetic endosperms modifiers are difficult to work with (Sofi et al. 2009). There are now significant opportunities for more effectively developing nutritionally enriched cultivars of both grain and specialty corn, thanks to various opportunities, including the supply of huge genetic diversity for the target traits,

Fig. 3 Soft endosperm o2 ears showing splitting of pericarp **Source:** Adapted from Nuss and Tanumihardjo, (2011)

advances in understanding key biochemical pathways for metabolite biosynthesis, analytical tools for screening germplasm for quality traits, and therefore the possibilities to utilize molecular markers and genome editing approaches to accelerate development (Reynolds et al. 2019).

Genetic background of QPM

To produce the QPM with the highly acceptable and recognizable lysine and tryptophan contents and thereby increasing the importance of QPM in Breeding and seed maintenance it is important that understanding the genetic background of QPM. QPM owes its origin primarily to a naturally occurring mutant, called *opaque2* (*o2*). Unlike Conventional Maize, in QPM the recessive allele has to be present in a homozygous state (o2o2) Fig. 4, which has a dominant allele at the same locus, usually in the homozygous state. Varieties derived from this original variant/mutant have been used throughout QPM development. In cases of incorporating the opaque2 gene into a CM background, the

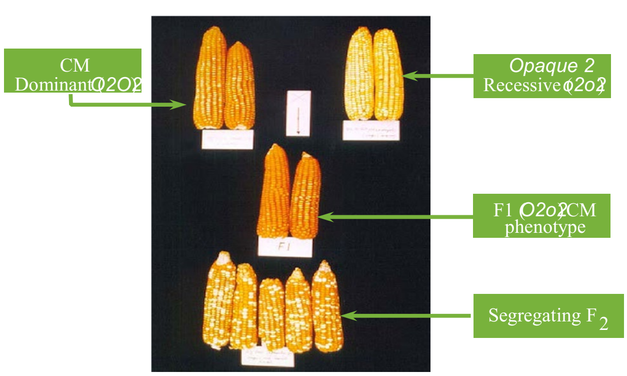

Fig. 4 Simple recessive inheritance of the the o2 gene. *Source:* Wegary, Ertiro and Bantte, (2015)

Scientists had employed conventional approaches in early time.

The QPM development involves manipulating the three distinct genetic systems (Bantte and Prasanna, 2003; Zunjare et al. 2018). a) the straightforward recessive allele of the opaque2 gene; b) Modifiers/enhancers of the o2-containing endosperm to confer higher lysine and tryptophan levels; and c) Genes that modify the o2-induced soft endosperm to hard endosperm. The opaque2 gene is that the central component of the genetic system that confers higher levels of lysine and tryptophan in maize endosperm protein. The allele is inherited in a simple recessive manner. The presence of opaque2 in the homozygous recessive (o2o2) state is a prerequisite for the entire process of obtaining high-lysine/tryptophan maize (Gibbon and Larkins, 2005). The presence of the opque2 allele in the recessive condition (o2o2) alone, however, does not ensure high lysine and tryptophan levels Table 1.

The second essential genetic system involves a group of genes that enhance the amount of lysine and tryptophan within the opaque2 genetic background. This genetic system consists of minor modifying loci (referred to as "amino acid modifiers") that enhance lysine and tryptophan levels in the endosperm (Li and Vasal, 2015a). Therefore, if lysine or tryptophan levels are not properly monitored while developing new cultivars, one could end up with a maize cultivar having the o2o2 genotype but with lysine and tryptophan levels similar to those in CM. This is because the lower limits of lysine and tryptophan in o2o2 maize overlap with the upper limits in CM (Li and Vasal, 2015a).

Phenotypic Selection for o2 Homozygotes with Modified Endosperm.

In the QPM breeding program segregating generations of homozygous o2 genotypes are selected visually. Both the presence of the o2 recessive mutation and that of the endosperm hardness modifier genes, which convert the soft, opaque endosperm to a hard, vitreous endosperm without much loss of protein quality, are selected through a simple, low-cost method of light-box screening, where seeds are placed on a Plexiglas surface above a light. Light is

Table 1 Lysine and tryptophan levels as percentages of total protein in whole grain flour of conventional and QPM (*o2o2*) genotypes**. Source:** Wegary, Ertiro and Bantte, (2015)

Traits	CM	QPM
Protein (%)	≥8	≥8
Lysine in endosperm protein (%)	1.6–2.6 (mean 2.0)	2.7–4.5 (mean 4.0)
Tryptophan in endosperm protein (%)	0.2–0.6 (mean 0.4)	0.5–1.1 (mean 0.8)

projected through the vitreous grains or blocked by the figure of the opaque grain. Individuals with opaque endosperm are considered to be o2 homozygotes. In an F2 population segregating for o2, breeders normally classify as o2 homozygotes those kernels that exhibit opacity at the base of the kernel but that are translucent above this area. The favorably modified endosperm of such kernels is considerably harder and more resistant to ear rots and insect damage than fully opaque kernels (Pswarayi and Vivek, 2008). This assay has the advantage that it can be used for single-kernel selection, but it is subject to person-to-person variability. It should be noted, however, that light-box screening results in some misclassification of putative o2 homozygote (Fig. 5).

Genotypic Selection for o2 Homozygotes with modified Endosperm.

The variation in levels of lysine and tryptophan in segregating families indicating the existences of the third set of genes that modify the amino acid content, which necessitates a systematic biochemical evaluation of lysine and/or tryptophan levels in each breeding generation, even if, o2 and associated endosperm hardness modifier genes are available. However, some major loci involved in o2 modification; for example, one locus map near the centromere of chromosome 7 and the second maps near the telomere on the long arm of chromosome 7 were revealed with help of genetic and molecular analyses (Dannenhoffer et al. 1995). In controlling the levels of a protein synthesis factor correlated with lysine levels at least three gene loci have been identified, the genes have been mapped to locations on chromosomes 2, 4, and 7. In the same genetic mapping studies, free amino acid content (including lysine) was measured using an alternative nine hydrin assay and nine significant loci were identified on chromosomes 1, 2, 3, 4, 5, 7, 8, and 9. As a result of these investigations, it has become apparent that the simple genetic nature of opaque-2 maize has transformed into a classic polygenic trait about QPM and must be manipulated as such in breeding programs. The additional gains in protein quality may be lost even though the o2o2 genotype is maintained if lysine or tryptophan levels are not continuously measured during the breeding process. Through efficiently monitoring the level of o2 and endosperm modifiers during the quality QPM breeding programs, therefore, it is possible to get favorable responses to selection for endosperm texture modification as well as the relative content of the essential amino acids (Gibbon and Larkins, 2005).

Fig. 5 Screening maize kernels on light table. *Source:* Vivek et al. (2008)

Nutritional benefit of QPM

QPM has relevant nutritional benefits for the people who depend on maize for their energy, and protein intake, and other nutrients. The opaque2 mutation is the basic source of QPM's nutritional benefits. Compared to CM, the QPM varieties, provide a more balanced protein for humans and other monogastric animals due to their higher lysine and tryptophan contents. It was firstly reported that the lysine content in o2 was 3.3 to 4.0 g per 100 g of endosperm protein, which was more than of normal maize endosperm (1.3 g lysine/100 g endosperm protein) (Crow and Kermicle,

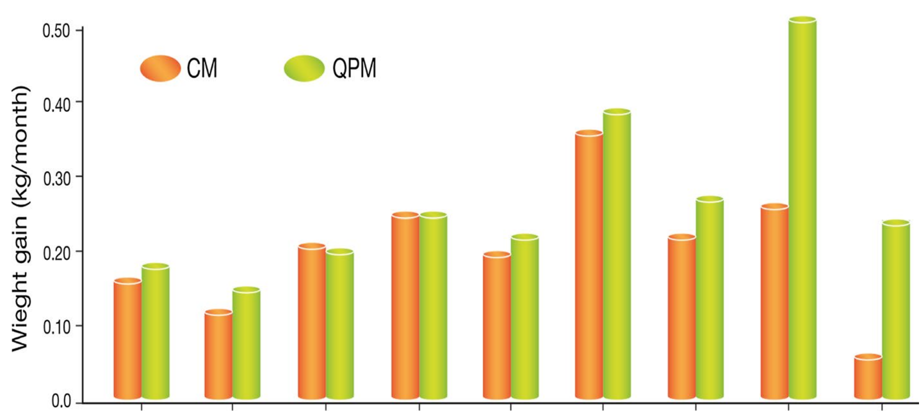

Fig. 6 Rate of increase in weight (kg/month) among children receiving conventional maize versus QPM. *Source:* Modified from Gunaratna et al. (2010)

2002). There is an overwhelming amount of data demonstrating the nutritional superior protein quality and protein digestibility of QPM over CM (Upadhyay et al.1970; Gunaratna et al.2010). In general, the QPM contains 55% more tryptophan, 30% more lysine, and 38% less leucine than that of normal maize as different studies demonstrated (Gunaratna et al. 2010). For people especially, who depend on maize for their energy, protein, and other nutrients, nutritional benefits are sufficient to justify its wide-scale production and promotion. In areas where participants, most often children are undernourished, several QPM feeding trials have been undertaken.

Another important additional factor of QPM is it is 'biological value' which refers to the amount of absorbed nitrogen needed to provide the necessary amino acids for different metabolic functions. Opaque-2 maize has 80% while normal maize protein has 45% biological value only. In terms of protein intake utilization, 74% of o2 maize protein utilizes while only 37% of common maize protein utilizes comparatively. Approximately, 125 g of o2 maize might guarantee nitrogen equilibrium from a minimum daily intake. Even a double-fold utilization amount of normal maize, could not bring this much of protein value (Li and Vasal, 2015a). QPM obtained 90% of protein quality than milk from the proportion of 0.80 o2 maize protein and 0.72 skim milk nitrogen balance index. Compared to QPM, around 24 g of normal maize per kg of the body is required for nitrogen equilibrium while only 8 g for QPM is required (Junaidu et al. 2015).

Malnourished children who were fed QPM as the only source of protein and fat recovered well and showed the same growth as those who were fed a modified cow milk formula (Qureshi et al. 2019). Gunaratna et al. (2010) investigated that combined analysis of various experiments carried out independently in different countries showed children consuming QPM instead of CM had a 12% weight increase. Overall, these studies concluded that consuming QPM improves growth rates and nitrogen metabolism, suggesting that it's going to be as efficacious as consuming casein, the milk protein (Gunaratna et al. 2010). Due to the significantly enhanced levels of tryptophan and lysine, it contains, QPM also reduces by half the quantity of maize that must be consumed to urge the same amount of biologically usable protein from a maize diet.

According to the report of (Ahenkora et al.1999; Graham, 1990) better leucine: isoleucine ratio; higher niacin availability; higher calcium availability when eaten in the form of lime-treated maize; higher carotene bio-utilization in yellow QPM; and higher carbohydrate utilization are another additional nutritional benefits of QPM. Independently conducted various experiments in different countries showed that children consuming QPM instead of CM had a 12% weight increase (Gunaratna et al. 2010). Out of these experiments, 9 of them proved strong shreds of evidence about nutritional benefits of QPM, while seven of these experiments showed that consuming QPM increased a child's weight as compared to CM Fig. 6.

The information collected from a focus group discussion in Sibu Sier Wereda revealed that the cooking quality and test of foods traditionally prepared from QPM were appreciated by the farmers. Due to its softness and longer shelf life, the injera prepared from QPM was preferred by farmers over that of CM. QPM porridge was also described as smoother than porridge prepared with CM. Mothers noted that QPM developed less of a sour taste when fermented than CM, making it more palatable to children. Children also liked the taste of "green" QPM grain over the taste of "green" CM because of its perceived sweetness; also, children did not feel hungry for a long time after consuming QPM-based food (Akalu et al. 2010b). The QPM is more acceptable and even preferred over CM for preparing widely consumed food products such as ugali in Tanzania, Githeri in Kenya, and injera in Ethiopia according to the experimental design conducted in eastern African countries indicated. De Groote et al. (2014) confirmed that these should be additional bonuses for farmers to produce and consume QPM and mitigate malnutrition, specifically in communities with poor quality protein intake and lysine deficiency, commonly associated with cereal-based diets.

In model systems in animals such as rats and pigs, the superiority of QPM toward nutritional and biological has also been amply demonstrated. For the first time in the feeding trials with rats, the superior quality of QPM protein was demonstrated (Cromwell et al. 1967; Salifu and Tindukin, 1882). More than three folds growth differences were observed between rats fed a diet of 90% QPM (97 g) over the rats fed CM (27 g). A study conducted by (Tiwari et al.2013) indicated that the pigs fed QPM demonstrated high differences than those fed CM Fig. 7. In general, significant growth changes were observed between pigs fed QPM and CM. Pigs fed a diet of QPM alone, except for vitamins and minerals, grew twice as fast as those fed CM (Vivek et al. 2008; Hossain et al. 2019). Chinese Academy of Agricultural Sciences (CAAS) undertaken a series of experiments on the nutritional value of QPM in poultry feed and pigs and proved the superiority of QPM over CM in terms of amino acid balance and nutrient composition, by improving the expansion and performance of varied animals. Diets processed with QPM are also more preferable, as they can lead to progressive reduction in the use of fishmeal and synthetic lysine additives (Qi et al. 2002) (Fig. 8, 9, 10).

QPM germplasm developed In Ethiopia

In developing countries, where maize is the dominant dietary source of energy and protein, a significant effort has been made to develop, release, and disseminate QPM varieties to address the issues of protein under-nutrition with technical and material support from CIMMYT and other organizations such as SG200. During 2003–2010 in four eastern Africa countries including Ethiopia, a lot of development agencies like Trade and Development (DFATD) (formerly the Canadian International Development Agency, CIDA), have supported the Quality Protein Maize Development (QPMD) project in QPM germplasm development and dissemination. The support from DFATD-Canada to Ethiopia has continued under the Nutritious Maize for Ethiopia (NuME) project since 2012.

1. With the evaluation of open-pollinated varieties (OPVs) and pools introduced from CIMMYT in 1994 the QPM development program was launched in Ethiopia with the main objective of fast-tracking the release of best-bet QPM varieties developed in several CIMMYT maize breeding hubs Wegary (et al. 2015) within the world. It was through this process that in 2001E.C. the first commercial QPM variety, BHQP542, was identified and released for commercial cultivation in the mid-altitude areas of Ethiopia. For the highland, mid-altitude, and moisture-stressed maize agro-ecologies of Ethiopia a full-fledged QPM development program was initiated subsequently, with the support from the QPMD project, with emphasis on the following:
2. Screening QPM varieties introduced from elsewhere for adaptation to local conditions. The introductions were either already commercialized in similar agro-ecologies in other countries or consisted of elite germplasm.
3. Conversion of popular and farmer-preferred CM cultivars into QPM versions. This strategy was aimed toward incorporating the opaque2 gene into parental lines of popular Ethiopian hybrids using the backcross breeding method. Parents of popular hybrids such as BH660 (A7033, F7215, and 142–1-e were used as recurrent parents, while proven CIMMYT QPM lines (CML 142, and CML176) were used as donor parents in the backcrossing program. F1 crosses were made between donor and recurrent parents to transfer the o2 gene from the donor to the recurrent parents. In the following season, F1 seeds were advanced to F2 by selfing the F1 plants to allow the expression of the target recessive gene. Using a light table, only F2 kernels that carried the o2 gene (i.e., kernels that were opaque to light) were selected and then crossed back to the recurrent parent (the parents of the CM). In subsequent years, three backcrosses were followed by advancing each backcross to the F2 generation, where selection for endosperm modification and monitoring the level of tryptophan were carried out regularly.
4. *QPM source germplasm development through mass conversion of elite non-QPM inbred lines or pedigree breeding with proven QPM lines*. Unlike the second approach, which targeted only parental lines of popular hybrids, this strategy aimed to convert a broad selection of elite conventional inbred lines into QPM versions through backcrossing Through repeated selfing of the F1 (obtained by crossing popular QPM parental lines) for 6–7 generations to select QPM inbred lines from the segregating progenies the pedigree method of inbred line development was used to develop inbred lines in addition to the above-mentioned method. Using the light table, kernels were selected for endosperm modification followed by tryptophan analysis after each selfing to identify promising QPM versions of the conventional inbred lines.

In close partnership with CIMMYT, the EIAR National Maize Research Program developed and released six QPM varieties using the three above-mentioned methods until 2014 for the three agro-ecologies of Ethiopia.

QPM varieties, their characteristics and adaptation

For commercial cultivation in different maize agro-ecologies of Ethiopia, six QPM varieties (four hybrids and two OPVs) have been released (Table 2).

Open-pollinated varieties (OPVs)

An OPV may be a genetically heterogeneous population maintained by open-pollination, which, when reproduced or reconstituted, retains some distinguishing features. The seed of an OPV is produced by random cross-pollination, i.e., there is no controlled pollination; instead, pollination occurs naturally without restriction within the population. Compared to hybrids have the following advantages:

- They are relatively easy to develop.
- The seed is straightforward and cheap to supply (it doesn't have distinct male and feminine parents and as a result, there's no need for detasseling).
- To reducing their dependence on external seed sources, farmers can save their seeds for replanting in the following season, although it is recommended that farmers purchase fresh seed every 3–4 seasons.
- They produce relatively lower yields and are not as uniform as hybrids.

Fig. 7 Pig fed QPM larger animal (Q4) compared with its sibling fed normal maize (N4). *Source:* adapted from Nuss and Tanumihardjo, (2011)

- They are not suitable for mechanized harvesting as compared to hybrids. EIAR National Maize Research Program had released two improved QPM OPVs for commercial cultivation in 2014, mainly for moisture-stressed maize agro-ecologies.

The names of the varieties and their target production zones are indicated below. Seeds of an OPV can be recycled with little or no yield penalty for a few (optimally three) years. However, it should be noted that tiny plots of QPM OPVs that are surrounded by CM fields are easily contaminated and hence won't maintain the specified protein quality.

- **Melkassa 6Q:** This OPV was released in 2008 for commercial production in moisture-stressed areas of the country. Under research management, its yield potential is about 4.5 to 5.5 tons per hectare (t/ha) and three 0.0 to 4.0 t/ha under farmers' conditions. on the average it takes 120 days to achieve grain maturity. thanks to its toler-

Fig. 8 Plant (Left photo) and ear (right photo) morphology of BHQP542. *Source:* Wegary, Ertiro and Bantte, (2015)

Fig. 9 Plant (left) and ear (right) morphology of BHQPY545. **Source:** Wegary, Ertiro and Bantte, (2015)

Fig. 10 *Plant (left photo) and ear (right photo) morphology of AMH760Q (webi). Please note the mixed tassel color that is characteristics of Webi.* **Source:** *Wegary, Ertiro and Bantte, (2015)*

Table 2 QPM released in Ethiopia and their agro-ecological adaptations, disease reactions and agronomic characteristics

Variety	Year of release	Adaptation	Plant height (cm)	Ear height (cm)	DM	Tassel color	Seed Color	Grain texture	Prolificacy	Yield (qt/ha)*		Disease reaction		
										RC	**FF**	**GLS**	**TLB**	**CLR**
BHQP542	2001	Moist midaltitude	220–250	100–120	145	Dark pink	White	Semi-flint	Prolific	80–90	50–60	T	MT	MS
Melkasa6Q‡	2008	Low moisture stress	165–175	70–75	120	White	White	Semi-flint	Non-prolific	45–55	30–40	–	T	T
BHQPY545	2008	Moist midaltitude	250–260	120–140	144	Pinkish	Yellow	Semi-flint	Highly prolific	80–95	55–65	T	MT	MT
AMH760Q	2011	Highland	240–290	143	183	50% white & 50% purple	White	Semi-flint	Prolific	90–120		T	S	MT
MHQ138	2012	Low moisture stress and moist midaltitude	200–235	100–120	140	White	White	Semi-flint	Prolific	75–80	55–65	T	T	MS
Melkasa1Q‡	2013	Low moisture stress	140–160	65–70	90	White	Yellow	Flint	Non-prolific	35–45	25–35	–	T	T

*1ton = 10 quintals (qt)

DM = days to maturity; RC = research center; FF = farmers field; T = tolerant; MT = moderately tolerant; MS = moderately susceptible; S = susceptible. **Source:** Ethiopian National Maize Research Program, EIR Wegary, Ertiro and Bante, (2015). All varieties except those followed by ‡ are hybrids

ance to low moisture stress during the flowering stage, this variety is popular within the Central valley areas of the Oromia and Southern Nations, Nationalities and Peoples (SNNP), Somalia Regions, and in some parts of Tigray. in several public and personal seed companies and farmers' cooperative unions, the seed of this variety is currently being commercially produced.

- **Melkassa 1Q:** this is often a QPM version of Melkassa 1 (a variety that's documented for its extra early maturity in areas with short rainfall duration and in marginal maize growing areas). Released in 2013, Melkassa 1Q is best suited to Melkassa 1's areas of adaptation and reaches grain maturity in just 90 days. Compared to other varieties Melkassa 1Q and its conventional counterpart aren't recommended for relatively high potential maize production areas thanks to their lower yield. At the research facility, the yield potential of this variety is 3.5 to 4.5t/ha and three 0.0 to 4.0 t/ha under farmers' conditions. Farmers who grow this variety should remember that it's exposed to bird and wild animal attacks due to its early maturity and short stature.

Hybrid QPM varieties

A product of a cross between two unrelated parents, one of which is designated as female and the other as male is called a hybrid. There are different ways to develop hybrids; 1) A single cross hybrid; is developed by crossing two different inbred parents. 2) A three-way cross hybrid; is formed from a cross of one inbred line with a single-cross hybrid parent. 3) Double-cross hybrid; formed by crossing two different single-cross parents and top-cross hybrids (formed by crossing an OPV to a single-cross hybrid). An example of a single-cross hybrid is BHQPY545 obtained by crossing two QPM inbred lines: CML161 (the female or "seed" parent) and CML165 (the male parent). BHQP542, MHQ138, and AMH760Q are an example of three-way cross hybrids.

Compared to OPVs these hybrids have more advantages; they produce higher grain yield and they have more uniform characteristics (particularly single-cross hybrids), making them more suitable for mechanization. In another way, these hybrids have also constraints comparing with OPV; they are more expensive, the price of hybrids seed is higher and the Farmers must purchase fresh F1 seed every year as the use of F2 results in a yield reduction of as much as 30% compared to F1 seed.

BHQP542 (Gabissa): A good adapted to the country's mid-altitude, sub-humid maize agro-ecologies (1000-1800 m.a.s.l.) QPM was released in Ethiopia in 2001. It has comparable grain yield and shares the same adaptation zones with BH540. This hybrid all developed by CIMMYT is a three-way cross hybrid involving three QPM inbred parents. This variety has several characteristics that have limited its adoption by farmers, including high susceptibility to common leaf rust, especially when grown in hot spot rust areas like Hawassa; susceptibility to turcicum leaf blight (TLB); and small kernel size (farmers see this as both an advantage and a disadvantage; when sold by volume, lower packing volume results in greater weight per unit volume and a lower price; however, farmers also report greater resistance to weevils due to the closer packing of kernels). Nevertheless, this variety has performed well in certain niches of the country, like Hadiya and Baduwacho in SNNPR, and Illuababora in Oromia.

BHQPY545 (*Kello*): In low- and mid-altitude sub-humid agro-ecologies the yellow kernel single-cross QPM hybrid was released in 2008 for commercial cultivation. This hybrid has been released in several countries globally where it enjoys wide popularity and is derived from two CIMMYT QPM inbred lines, CML161 and CMLL165. This variety is high yielding, lodging resistant, and early maturing performances in addition to it is a nutritional advantage. Usually, it bears two or more ears per plant under good management. Average yields of 8.0 to 9.5 t/ha on the research station and 5.5 to 6.5 t/ha under farmers' conditions have been recorded. On the research station, the average yield of this variety is 8.0 to 9.5 t/ha and 5.5.to 6.5 t/ha under farmers' conditions. Some farmers have managed to produce up to 9.8 t/ha of grain in farmer-managed demonstration plots in the Gobu Seyo district in East Wollega. The demand for BHQPY545 is high due to the following reasons, though consumers prefer maize with white kernels.

- Particularly for children, as well as pregnant and lactating women, it increased awareness in the community of the nutritional advantage of the variety. Its elevation with provitamin 'A' *contents* is another preference of the nutritional factor of yellow kernel color.
- Because of its yellow color, demand from the country's flourishing poultry industry for BHQPY545 is highest to reinforce ingredient color and protein quality (to supplement protein in rations).
- It's suitable for green ear consumption, both in taste and prolificacy.
- For creating corn flakes, yellow maize like BHQPY545 is most preferable.
- Farmers in Bako Tibe, Illu Gelan, Gobu Seyo, and Sibu Sire districts who cultivated this variety have received premium farm prices from the FAFA food processing factory recently.
- Due to open ear tips under conditions of high fertility, this variety is low to moderately suffering from ear rot.
- To reduce the incidence of ear rot, growers are advised to use one among the subsequent strategies:
- Avoid growing this variety in areas where ear rot is prevalent.
- Since it's prolific under optimum management conditions, cultivated the variability for the green ear market. As a reason of ear rot are favorable by excessive moisture penetrating the ear, delay planting the variety so that will mature late in the season when rainfall is subsiding or has ended.
- Grow the variety during the off-season under irrigation in areas to which it is adapted, thus avoiding excessive moisture as the crop matures.

AMH760Q (Webi): A three-way hybrid cross adapted to the highland agroecology of Ethiopia (1800–2600 m.a.s.l) converting the parental lines of BH660 into QPM through the backcross breeding method was released. Webi was produced by a program aimed at developing QPM varieties that are competitive with BH660 in terms of grain yield in the transitional and highland areas. The variety is adapted to highland areas such as Ambo, Kulumsa, Adet, Guder, and Gudeya Billa.

Webi has some weaknesses and certain peculiar features that a grower should remember of:

• Farmers in highland areas where TLB is a serious problem are advised to grow other QPM varieties with tolerance to the disease since the Webi is susceptible to turcicum leaf blight (TBL).

Comparing with BH660 which is uniformly purple, Webi has mixed purple and white (50:50) tassel as a varietal characteristic. As a result, this mixed tassel color does not indicate seed contamination and does not affect grain yield. If the proportion of purple and white tassels in Webi deviates significantly from 50:50, however, it could be due to contamination.

MHQ138: MHQ138 QPM hybrid was developed through a three-way cross for moisture-stressed areas of the country. To areas with higher rainfall such as the moist mid-altitude agro-ecologies, it is also well adapted. Because this variety has the same female as BHQP542 (CML144/CML159), and its male parent is from POOL15Q, it matures somewhat earlier than BHQP542 and BH540. MHQ138 is tolerant to drought and adapted to dryland areas such as the Central Rift Valley and the northern, eastern, and southern parts of Ethiopia. On-farm demonstration plots in the vicinity of Bako it has shown higher yield potential than when it is grown in a moisture-stressed area. Therefore, in high potential transnational midland areas, this hybrid could be used as an alternative QPM variety because of it is earliness.

Conclusion

Although a significant achievement was celebrated, the agricultural production challenges to feed the ever-increasing population is still among a major issue of the world. In the face of rapid population growth and recurring natural disaster in 1950, the world needs a large boost in food production to combat famine. Providing a nutritionally balanced diet and producing enough to feed the growing population has been seen as major challenges in the twenty-first century. The food today on everyone's tables must be enough quantity, nutritious, and produced in an environmentally, economically, and socially sustainable manner despite huge discussion on calorie security. The importance of promoting diets that are nutritious and which can reduce the environmental impact of food systems has been recommended by several food commissions. Any strategies of agriculture and nutritious sensitive strategies developed to minimize food security and malnutrition could contribute to a sustainable and nutritional food system. To successfully develop and release several nutritious maize cultivars without compromising grain yield levels or other important agronomic and adaptive traits, CIMMYT, IITA, and national partners have employed conventional breeding and molecular tools especially in Africa, Asia, and Latin America. In many countries, these biofortified maize cultivars are currently grown by farmers and accepted by consumers.

Considerable hybrid development efforts in QPM have progressed by several national maize programs. Soon, those countries involved in the QPM network expected to select some of the most promising hybrid combinations developed by CIMMYT for release than they have done before. Amongst several efforts that have been done for conversions of elite local inbred lines into QPM versions particularly those developed by CIMMYT was achieved successfully. Particularly in the developing countries, however, there is still a shortage of widespread development and deployment of QPM cultivars. It has to be known that in such developing countries, maize produced by small farmers is mainly for own consumption and save seed for subsequent sowing. Thus in such areas, it is fact that it is difficult to introduce and effectively exploiting high-yielding QPM hybrids may be difficult. Nevertheless, even under the small farm, there is the hope that public sector institutions can bring a considerable increase in QPM adoptions. A successful deployment of QPM hybrids in Africa countries such as Ghana and Ethiopia is made a good benchmark for future successions. Lack of adequate funding, trained scientific and technical personnel, knowledge of the possible benefits to be derived from high-quality protein maize are major identified constraints to be solved for the future. In countries economically deprived regions where maize is used for food and feed purposes, huge efforts are required for better public awareness and dissemination of QPM technology.

Achievements recorded for the breeding targets of various nutrients in maize were a result of advances in phenotypic coupled with molecular breeding facilitations. To deliver high-performing climate-resilient maize cultivars with improved nutritional quality for farmers and consumers should be mainstreaming breeding for nutrient enrichment into maize is a future needed breeding efforts. Globally, it is important to develop and deploy improved, low-cost, affordable QPM hybrids, with higher grain yield and quality to provide better food and nutrition security of maize-based population to meet nutritional requirement among resource-poor people. In QPM research, there will be an increasing application of molecular genetic tools in the coming year. To the end, this review will exploit and facilitate the status of QPM, genetic background study for scholars working on QPM matter and promote the development of QPM for countries that have a direct relation with maize for food security.

Acknowledgements I would like to thank the CERC Agriculture and Bioscience Journal Reviewers.

Funding "Not applicable" in this section.

Data availability All data mentioned in this paper was acknowledged. Table, figures and map used in this paper was adapted from different papers.

Declarations

Conflict of interest The authors declared that they have no competing interest.

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