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Review

Breeding for Quality Protein Maize (QPM) Varieties: A Review

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Abstract: The nutritional evaluation of quality protein maize (QPM) in feeding trials has proved its nutritional superiority over non-QPM varieties for human and livestock consumption. The present paper reviews some of the most recent achievements in development of QPM varieties using both conventional and molecular breeding under stressed and non-stressed environments. It is evident that numerous QPM varieties have been developed and released around the world over the past few decades. While the review points out some gaps in information or research efforts, challenges associated with adoption QPM varieties are highlighted and suggestions to overcome them are presented. The adoption of released varieties and challenges facing QPM production at the farmer level are also mentioned. Several breeding methods have been conventionally used to develop QPM varieties in stressed (drought, low soil nitrogen, resistance to grey leaf spot, *Turcicum* leaf blight, ear rot, and Striga) and non-stressed environments. At least three genetic loci have been found to be implicated in controlling the levels of a protein synthesis factor correlated with lysine. They have been mapped on chromosomes 2, 4, and 7. While the use of molecular approaches will improve the efficiency and speed of variety development, the cost implications might limit the use of these technologies in the developing world. More emphasis should be given to breeding QPM for tolerance to environmental stresses, such as low soil pH, heat, and combined heat and drought stress. The post-harvest attack of QPM grains should also be considered. The adoption of QPM genotypes by farmers has been found to be limited mainly due to the minimal collaboration between maize breeders, farmers, agricultural extension workers, and other relevant stakeholders, as well as the need for isolating QPM varieties from normal maize. Therefore, there is need to use participatory plant breeding (PPB) and/or participatory variety selection (PVS) to enhance and improve the adoption of QPM varieties.

Keywords: QPM; breeding; adoption; PPB; PVS

1. Introduction

Maize (*Zea mays* L.) is the third major cereal crop in the world after wheat and rice and is used for both livestock feed and human consumption [1]. The crop also has other industrial and non-industrial uses. Maize contributes 15% of the world's protein and 19% of the calories derived from food crops [2]. Millions of people in the world, and particularly in developing countries, derive a part of their protein and daily calorie requirements from maize [3]. The crop is also an important component of livestock feed, especially in developed nations where 78% of total maize production is used for livestock feed [4]. In Africa, maize supplies at least one fifth of total daily calories and accounts for 17% to 60% of the total protein supply per day of individuals who are more susceptible to risk of protein or essential amino acid deficiencies [5]. Pregnant women, lactating mothers, and young children are particularly the most affected [2,6]. To alleviate malnutrition, protein content in maize can be increased to as high as 18% (close to double the quantity of protein in normal maize) by increasing the prolamine (zein) fraction in the maize endosperm [7].

Quality protein maize (QPM) was developed in the late 1960s [1] and it produces 70% to 100% more lysine and tryptophan than ordinary modern and traditional varieties of tropical maize [8]. Additionally, nutritional evaluation of QPM in various locations has proved the stability of lysine and tryptophan content within the prescribed range for QPM, in spite of quite diverse types of environmental conditions [9]. The nutritional quality of the protein in QPM grain approaches that of protein derived from cow's milk [1]. The adoption of QPM can contribute immensely to alleviation of malnutrition in maize-based economies in developing countries [10]. For instance, it has been found to be of economic value to substitute normal maize in stock feeds as it requires small amounts or no supplementary protein sources to balance the diet [11,12].

Research on QPM has been ongoing for several decades. Opaque-2 (o2) is a natural recessive mutation in the transcriptional activator conditioning negative expression of zein protein [13]. However, the lower yields of QPM versus non-QPM varieties, as well as the susceptibility of QPM varieties to stresses, such as ear rot, resulting in less tryptophan and lysine produced per unit area of land have been the focus of researchers over a number of years. Nowadays, despite the nutritional differences, some QPM varieties are as productive as non-QPM and sometimes it is difficult to visually distinguish between the two types of maize by the physical appearance of the plants or the ears. QPM genotypes can produce yields as high as the non-QPM varieties (Table 1). However, normal maize generally yields more [14], though in some situations the difference with QPM genotypes can be marginal. The need for biochemical analyses to determine the lysine and tryptophan content of the grains to confirm whether or not it is QPM has always been emphasized [15].

Table 1. Yield range of QPM and non-QPM genotypes.

Type of Variety	QPM Yield Range (t/ha)	Non-QPM Yield Range (t/ha)	Reference
OPVs and synthetics	2–7.3	>4	[14,16]
Hybrids	3–13.9	>5	[1,14,17–19]

QPM cultivars could be competitive with normal maize in productivity and should show stable performance across environments, especially with respect to yield and protein quality traits [9]. It has been a great challenge to combine high yield with high-quality protein content in an elite maize variety. As with normal maize, QPM production faces serious biotic (diseases and pests) and abiotic (drought, heat, low soil pH, low soil nitrogen, etc.) constraints. To alleviate some of these constraints, several studies have been done around the world on breeding for the resistance of QPM to pests and diseases [14,16,20,21] and tolerance to abiotic stresses such as drought, low soil nitrogen, heat stress and, combined heat and drought stress [9,14,21–28].

Quite a number of studies have been conducted on QPM regarding the improvement of the nutritional value and diseases resistance of QPM over the past years in breeding programs around the world [13,29–34]. Increasing the protein content and investigating genetic variability among QPM genotypes and normal endosperm maize varieties have been the major research focus. However, more research needs to be placed on the tolerance of QPM varieties to some abiotic stresses, such as heat stress, combinations of drought and heat stress and low soil pH. Given the superior nutritional characteristics of QPM varieties, this paper presents the latest information on conventional and molecular breeding methods that have been applied to develop QPM genotypes. It gives an overview of QPM varieties released worldwide, the adoption and challenges facing QPM production at farmer level. While some gaps in information and research efforts are highlighted, suggestions that could improve adoption of QPM varieties are also presented.

2. Nutritional Quality and Impact of QPM

Malnutrition is a persistent problem in Africa, especially in rural areas where poor people depend on staple foods and have limited access to a diverse diet. Bio-fortified crops bred for improved nutritional quality can alleviate nutritional deficiencies if they are produced and consumed in sufficient quantities [35]. Several studies in controlled settings have indicated the positive impact of QPM on the nutritional status of human consumption and animal feeds [12,34,36–41].

A natural, spontaneous, recessive mutation of maize with soft opaque grains was first described by Jones and Singleton in the early 1920s [42]. However, the nutritional significance of the mutation was first discovered by Mertz and coworkers [29,43]. In 1963, researchers at Purdue University in USA discovered that opaque-2 (*op2*) created grain proteins in the endosperm nearly twice as those found in normal maize [29]. Endosperm of maize grain is the store house of storage proteins [13,31]. Homozygous maize varieties with two copies of the mutation (recessive *o2* allele) have substantially higher lysine (69%) in grain endosperm compared to normal maize [29]. The proportion of lysine and tryptophan in the total portion of protein were found to be almost double in QPM materials (4.1% and 1%, respectively) than in non-QPM (2.7% and 0.6%, respectively) (Table 2).

Table 2. Proportion of lysine and tryptophan in the total protein of non-QPM, QPM material, and the recommended protein proportion for children and adults.

	Total Protein	Lysine	Tryptophan
QPM (%)	>9	4.1	1
Non-QPM (maize flour)	>9	2.7	0.6
FAO recommendations (g/100 g total protein)			
Children		6.6	1.7
Adults		1.6	0.5

Adapted from [12,44].

Additionally, QPM also showed a corresponding increase in tryptophan content, which doubles the biological value of ordinary maize protein [36]. To classify the maize grain as QPM, the quality index, which is the tryptophan-to-protein ratio in a given sample, must be higher or equal to 0.8% [34]. The quality index of hybrids derived from crosses between QPM lines and normal commercial lines was in the range of 0.71 to 0.74, which was better than in standard hybrids (0.57–0.62) but below the QPM threshold (0.80) [33]. Consumption of QPM could go a long way in reducing the growing challenge of malnutrition in various parts of the world [33,36,37,39,41,45,46]. The nutritional benefits of QPM have been demonstrated numerous times in feeding experiments involving livestock [5,41] and children [37]. For instance, children who ate porridge made from QPM had fewer sick days relative to those who ate porridge from common maize (CM). Consumption of QPM instead of CM resulted in a 12% increase in the rate of growth in weight and 9% increase in the rate of growth in height in infants and young children with mild to moderate under nutrition [39]. A study in some African countries including Lesotho, Malawi, and Zambia showed that around 100 g QPM is required for children to maintain adequacy of lysine (which is the most limiting amino acid) representing, a 40% reduction in maize intake requirements relative to CM [40]. QPM germplasms have different maturity periods, as well as different grain color and texture [31]. Some QPM varieties have high levels of carotenoids [45], which are a precursor of vitamin A [46].

Ignjatovic-Micic et al. [33] screened 72 hybrids (derived from crosses between QPM lines adapted to temperate environments and three commercial lines with normal kernel quality) for kernel modification, tryptophan and protein contents, quality index, and grain yield. The percentage of kernel modifications was over 80% in three hybrids and over 74% in two hybrids, thus showing the complexity of obtaining high yielding hybrids with high levels of essential amino-acids using their crossing method. Forty-five single-cross hybrids developed from ten inbred lines of QPM and four checks (Prata QPM hybrid, Vivek QPM-9, HQPM-1, and HQPM-5) were evaluated in 2014 for yield and nutritional quality and the result showed that six crosses significantly out-yielded the standard check, whereas 42 expressed mid parent heterosis, while 37 over yielded the best parent [18]. Akalu et al. [38] studied the effect of QPM on the nutritional status of children consuming typical maize-based diets when QPM was cultivated by their households in the western Ethiopian highlands and found a significant effect of QPM in the weight gain of children and concluded that the use of QPM in children's diets could reduce or prevent poor growth and may in some cases support catch-up growth in weight. Consuming opaque-2 maize improves growth rates and nitrogen metabolism, suggesting that it may be as efficacious as consuming milk casein [40].

The levels of lysine or tryptophan should be continuously measured during the breeding process to maintain the protein quality in grain even when the *op2op2* genotype is maintained. The consumption of QPM varieties is one way of solving the problem of malnutrition in the world, especially for people poor in resources who cannot afford other sources of protein for their families. QPM can also be used as supplementary food for humans (pregnant women, lactating mothers, and young children, in particular) and to feed animals. Moreover, QPM flour can also be very useful for refugees and other people facing nutritional problems around the world.

3. Breeding Approaches

After the discovery of the nutritional benefits of the opaque-2 (*op2*) mutation, it has been incorporated into many breeding programs worldwide, with a major emphasis on conversion of normal endosperm populations and inbred lines to *op2* versions through a modified back crossing–cum–recurrent selection method [1]. The soft endosperm of *op2op2* genotypes initially caused up to a 25% yield loss due to the lower density of the opaque grains, as well as increased susceptibility to fungal ear rots and storage pests [47]. The soft endosperm texture was also not accepted in the developing world accustomed to harder grain types [5]. Initial QPM breeding efforts at CIMMYT focused on conversion of a range of subtropical and tropical lowland adapted, normal endosperm populations to *op2* versions through backcross-recurrent selection procedures, with a focus on accumulating the hard endosperm phenotype, maintaining protein quality, and increasing yield and resistance to ear rot [5,48]. Basically, two approaches were used in developing QPM donor material: the first approach was intra-population selection for genetic modifiers in opaque-2 backgrounds exhibiting a higher frequency of modified *op2* kernels and the second approach involved recombination of superior hard endosperm opaque-2 families [31]. QPM breeding strategies at CIMMYT focus on pedigree breeding whereby the best performing inbred lines, complementary in different traits, are crossed to establish new segregating families. New inbred lines are developed from these segregating families [5]. The *opaque-2* mutation is reported to be associated with numerous modifiers which together behave as a polygenic trait [13]. In general, QPM materials had 10–15% less grain yield with the kernel appearance being dull and chalky, slower drying, as well as the greater kernel rots and greater vulnerability to stored grain pests [2]. Several research studies have been done over the years to solve these constraints on QPM varieties. Different methods have been used so far in the improvement of QPM breeding programs: conventional and molecular approaches.

4. Conventional Breeding Approaches

4.1. Screening of QPM Genotypes for Abiotic Stresses

Pixley and Bjarnason [15] reported that protein quality was very stable across environments, whereas protein content and endosperm modification of QPM varieties were less stable. Pfunde and Mutengwa [17] found that early maturing QPM inbred lines under drought stress such as CML18 could be used as sources of earliness in a breeding program, whereas early maturing single crosses, such as CML18 × CML2, CML5 × CML9, CML3 × CML4 and CML2 × CML21, could potentially be recommended for maize growers in drought-prone areas, like the former Ciskei in the Eastern Cape province of South Africa.

Zaidi et al. [9] studied the stability performance of CIMMYT tropical and subtropical elite QPM hybrids across stressed (drought at flowering stage and low soil N) and non-stressed environments and found that the variation in protein quality across environments was statistically significant, but was largely due to genotypic variability. Additionally, stress significantly affected all agronomic traits except male flowering period whereas, among the quality traits, grain protein, tryptophan, and lysine contents showed significant variation across environments with an increase in grain protein (+12.7%) and in lysine (+10.3%) and tryptophan contents (+8.1%) under drought stress, while levels of these grain quality traits were reduced by (−17.0), (−12.5), and (−15.6%), respectively, under low N.

An evaluation of 49 QPM single cross hybrids generated using a North Carolina Design II during the 2015/2016 season under combined drought and heat stress conditions in the Eastern Cape province of South Africa showed that cross combinations CML3 × CML13 (3.05 t/ha) and CML5 × CML9 (2.95 t/ha) were the best performing single cross hybrids under the combined drought and heat-stressed environment [17].

The evaluation of 85 QPM hybrids under well-irrigated and water-stressed conditions showed that four of them were more drought-tolerant based on their response to drought tolerance indices [24]. Moreover, the results of correlation, 3D graphs, bi-plot and cluster analyses revealed that the most suitable indices to screen QPM genotypes in drought stressed conditions were mean productivity, geometric mean productivity, and yield index [24]. These indices are suitable when screening for late drought stress occurring at flowering and grain filling stages but not for early drought (seedling stage).

Zaidi et al. [9] analyzed the stability performance of CIMMYT tropical and subtropical elite QPM hybrids across stressed (late drought and low N) and non-stressed environments. The stress effect was comparatively large under drought conditions with large variability on the grain yield and protein content among genotypes, suggesting that tryptophan and lysine content are most stable across stressed and non-stressed environments.

While screening for drought tolerance has largely been performed for the vegetative to flowering stages of growth for QPM varieties, very few investigations on tolerance to early drought stress at the seedling stage have been done. Drought tolerance at the seedling stage of growth has sometimes been despised in that it does not provide an indication of the yielding capacity of a genotype under drought stress. There is, therefore, clearly a need to further investigate the correlation parameters which could link early drought response to late drought stress tolerance. Even though the critical period of drought effect on QPM production has been found to be at the flowering and grain-filling stage, the early drought occurrence could negatively affect plant density, resulting in lower yields. Therefore, early drought occurrence should be taken into consideration. While several studies have been investigated the tolerance of QPM to drought and low soil nitrogen, other environmental stresses such as low soil pH, salinity, heat stress and combined drought and heat stress have generally received very limited attention. Developing high-yielding QPM genotypes across the stressed and un-stressed environments should continuously be the target of breeders.

4.2. Screening of QPM Genotypes for Tolerance to Biotic Stresses

The performance of high–yielding QPM hybrids recorded across 41 locations in Latin America and Asia from 1999 to 2000 showed grain yield ranging from 5.6 to 6.4 t/ha with 5.5% to 7.8% variation for ear rot, with a tryptophan content of up to 10% (Table 3).

Table 3. Performance of superior tropical QPM hybrids and one check across 41 locations in Latin America and Asia from 1999 to 2000 [2].

Pedigree	Grain Yield (t/ha)	Ear Rot (%)	Silking (50%)	Endosperm Hardness *	Tryptophan (%)
CML-141 × CML-144	6.4	5.5	55	1.6	8.8
(CML-141 × CML-144) CML-142	6.4	6.2	55	1.7	8.1
CML-142 × CML-146	6.3	6.3	55	2.2	10
CML-142 × CML150	6.2	7.8	55	2.0	8.9
(CML-142 × CML150) CML-176	6.1	7.5	55	2.0	8.6
CLQ-6203 × CML-150	5.8	7.2	55	2.3	9.0
CML-144 × CML-159	5.9	5.9	56	1.9	9.3
(CML-144 × CML-159) CML-176	5.6	6.0	56	1.7	9.4
Local check	5.9	7.6	55	1.9	5.0

* Rating scale: 1–5 with 1 = completely hard and 5 = completely soft.

Some QPM maize streak virus resistant lines developed at CIMMYT were distributed to the National Agricultural Research System (NARS) in Uganda (Namulonge), Tanzania (Selian), Ethiopia (Nazareth, and Bako), and Kenya (Embu) for further breeding work in 2002 [14]. The evaluation of some QPM germplasm under both stressed and non-stressed conditions in Kenya showed that QPM OPVs expressed grain yield of around 2 t/ha under a combination of diseases (grey leaf spot, *Turcicum* leaf blight and ear rot) (Figure 1b) and low soil nitrogen stress (Figure 1a). However, a non-QPM hybrid had the best yield under optimum conditions and low soil nitrogen compared to all QPM materials.

Sixteen early and nine extra-early-maturing QPM and non-QPM genotypes were evaluated under *Striga hermonthica* (Del.) Benth infestation and *Striga*-free environments in Nigeria from 2006 to 2008 [22]. Results showed that TZE-W DT STR C_4 (normal maize) out-yielded the QPM version by 21% under *Striga* infestation and by 10% under *Striga*-free conditions, whereas the QPM cultivar 98 Syn WEC STR QPM C0 out-yielded the normal endosperm version by 31% under *Striga*-infested conditions. The extra-early normal maize cultivars, 2000 Syn EE-W STR and 99 TZEF-Y STR, were similar in yield to the QPM version under both research conditions. While TZEE-Y Pop STR C 4 was superior in grain yield to its QPM version only under *Striga*-free conditions, TZEE-W Pop STR C4 significantly out yielded the QPM version, under both test conditions and was superior in *Striga* resistance [22].

QPM varieties have the potential to resist the attack of some diseases (such as grey leaf spot, maize streak virus, ear rot, Turcium leaf blight) and pests such as *Striga*, but there is a need of conducting more investigations, especially for QPM resistance to post-harvest pest and disease attacks of the grain, which could have positive impacts on the utilization and adoption of QPM varieties by consumers and growers.

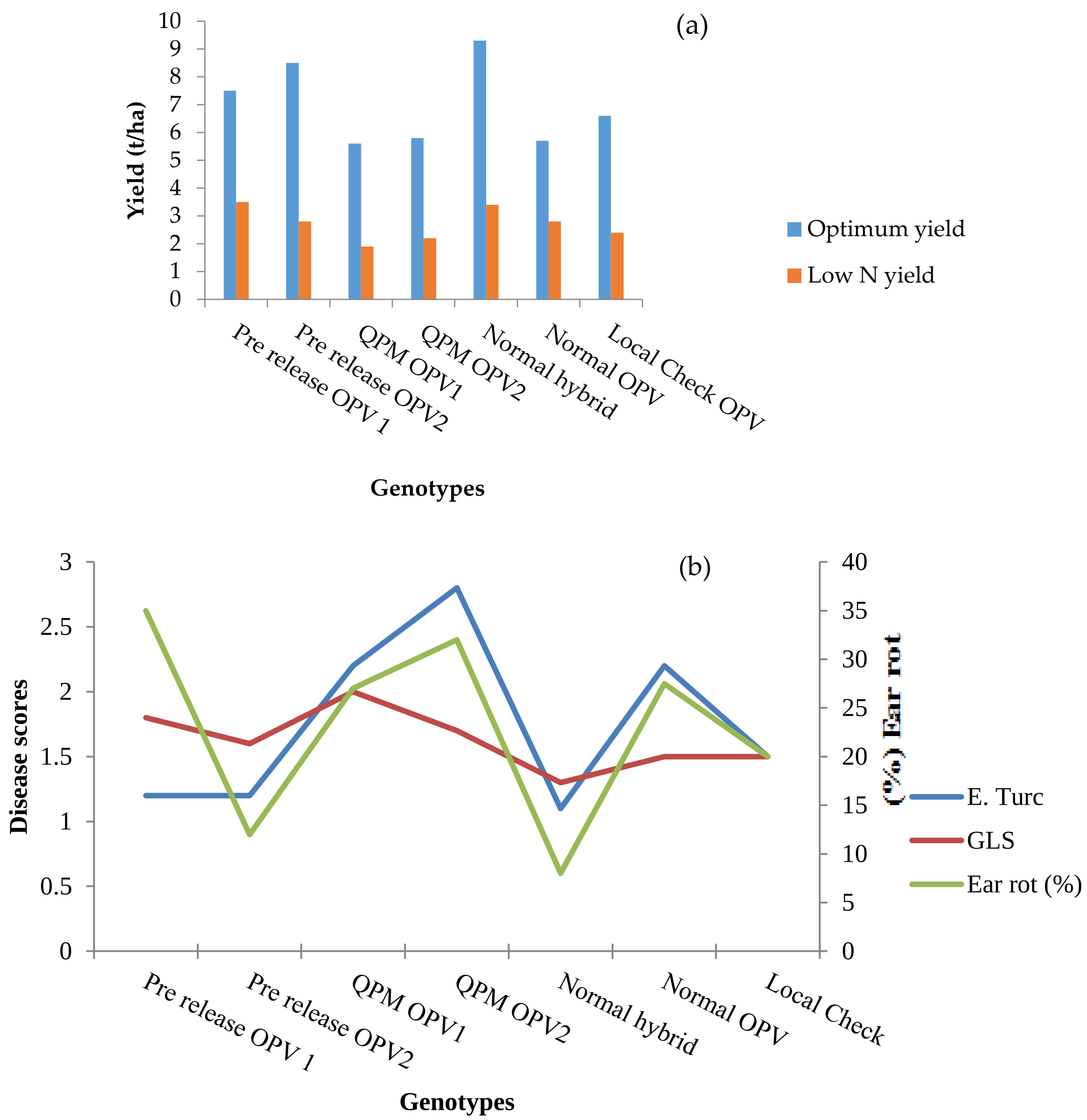

Figure 1. Grain yield (t/ha) (**a**), and disease (**b**) (GLS, *Turcicum* leaf blight and ear rot) reaction of two QPM OPVs, two prerelease QPM hybrids, two normal OPV_S and one normal hybrid, tested in 2003 across four sites including low N and optimum soil conditions in Kenya (adapted from [14]).

5. Combining Ability Studies of QPM Genotypes Traits for Tolerance to Biotic and Abiotic Stresses

Several studies have been conducted on the combining ability effects of QPM genotypes under normal and stress environments. In some cases, the traits recorded were conditioned mainly by additive gene effects under stress conditions (drought, low soil nitrogen, heat stress, combined drought and heat stress, *Striga*) and non-stress environments [20,21,23,26–28,49]. Evaluation of yellow QPM genotypes under combined drought and heat stresses showed that grain yield was influenced by non-additive gene effects in South Africa [27]. QPM hybrids evaluated under drought, low soil N, and *Striga* (*Striga hermonthica*)-infested environments in Nigeria indicated that non-additive gene action largely modulated inbred trait inheritance [26]. Some combinability studies conducted in QPM breeding over the years are presented in Table 4.

Table 4. Combining ability studies for QPM genotypes evaluated over the years.

Plant Material	Environment	Combinability	Reference
Hybrids and inbred lines	Ethiopia	GCA effects were larger than SCA suggesting the effects of additive genes.	[49]
Single-cross hybrids	Well-watered, drought stress and low nitrogen conditions in Eastern Africa	GCA effects were significant indicating the effects of additive genes.	[23]
Extra-early QPM single-crosses	Drought and low-N environments in Nigeria.	GCA effects contributed more suggesting the effects of additive genes.	[26]
Single hybrids	*Striga* infested, drought, low-N and optimal environments in Nigeria.	GCA effects were more important suggesting the effects of additive gene action.	[20]
QPM hybrids	Drought, low soil N, and *Striga* infested environments in Nigeria	SCA effects were higher than GCA indicated the action of nonadditive genes.	[21]
Inbred lines and single cross hybrids	Combined drought and heat stresses in South Africa.	Grain yield was mostly controlled by non-additive gene action.	[17]
Single cross hybrids	Combined drought and heat stress in the Eastern Cape province of South Africa.	Additive genetic effects influenced most traits except grain yield, which was influenced by non-additive gene effects.	[27]
QPM hybrids	Rainfed condition in Ethiopia	Larger addidive genetic variation for grain yield.	[50]

GCA: General Combining ability; SCA: specific combining ability.

In breeding, grain yield is the most important quantitative trait which is controlled by multiple genes. The knowledge of the gene actions and their relative contribution in the expression of the trait is very important in breeding process. The non-additive gene actions involved the dominance and epistatic effects of genes for inheritance of the trait. It is difficult to select for yield directly when controlled by non-additive gene action. However, selection for yield under stress conditions can be effectively accomplished through its yield components, which are generally controlled by additive gene action. Therefore, breeding for high-yielding QPM hybrids should be of interest with emphasis put on the establishment of heterotic groups. GCA is due to genes which are largely additive in their effects. In this case, it is possible to successfully select better individuals in segregating populations. Therefore, the improvement of elite QPM varieties could be done through back cross selection.

6. Molecular Approaches

The molecular structure of the opaque-2 gene was investigated by Henry et al. [51] who found that the molecular diversity in the *op2* transcriptional activator was quite high compared to that of other transcription factors in maize. Multiple genes have been identified in controlling amino acid content. At least three loci have been implicated in controlling the levels of a protein synthesis factor correlated with lysine levels and these have been mapped on chromosomes 2, 4, and 7 [52–54].

CML 180 and CML 170 were selected as QPM donors for introgression of the *op2* allele into normal maize inbreds CM 212 and CM 145 through marker assisted backcross breeding because the crosses between the donor QPM lines and non-QPM lines expressed 41% increase in tryptophan and 30% increase in lysine over the original hybrid [51]. Maize accessions from the DR-Congo breeding program were analyzed using ISSR primers and variety-specific diagnostic markers were identified and characterized. The development of an ISSR diagnostic marker indicates the possibility of developing a molecular breeding program involving QPM and non-QPM varieties [55]. Modified marker-assisted back cross breeding is, therefore, a possible way for the development QPM versions of normal maize inbreds with desirable endosperm characteristics which may be combined to develop QPM hybrids [51].

Nkongolo et al. [56] studied the level of genetic variation and relatedness among and within QPM and non-QPM varieties selected in the DR-Congo breeding program using inter-simple sequence repeat (ISSR) and random amplified polymorphic DNA (RAPD) markers. The results showed that the genetic variation among and within QPM and non-QPM varieties was high, whereas the genetic distance among them was small, giving the possibility of developing improved QPM hybrids.

Three simple sequence repeat (SSR) markers (phi057, phi112, and umc1066) used in the selection and introgression of the opaque-2 (*op*2) gene in maize genotypes in Uganda showed that the polymorphic SSR markers (phi057 and phi112) correctly predicted the expression of tryptophan in kernels of all QPM inbreds, as well as five of the six non-QPM inbred lines. These markers could, therefore, constitute the framework for marker assisted introgression of the *op*2 trait into maize genotypes [57]. Badu-Apraku et al. [20] grouped inbred lines based on GCA of multiple traits and the SNP-based genetic distance methods and they found that the two methods identified two groups each across research environments with close correspondence among the classifications of all the grouping methods in terms of placement of inbreds into the same groups. Moreover, the SNP-based method was found to be most efficient in identifying TZEQI 87 and TZEQI 91 as the best testers for the SNP-based heterotic groups 1 and 2. One of the main focuses of CIMMYT scientists is to develop reliable, easy-to-use markers for endosperm hardness and free amino acid content in the maize endosperm 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 QPM maize to enhance efficiency and cost effectiveness of MAS in QPM breeding programs [58]. Proteomic analysis has been also studied in QPM breeding [28,59,60]. Some case studies conducted on QPM genotypes using molecular approaches are summarized in Table 5.

Table 5. Different molecular methods used for QPM breeding investigations.

Technic	Plant Material	Aim	Reference
Simple sequence repeat markers (SSR)	QPM and non-QPM	Marker assisted introgression of the o2 trait	[57]
ISSR primers and variety-diagnostic markers	QPM and non-QPM	Molecular breeding program development	[55]
SDS-PAGE	QPM grain	Protein identification	[59,60]
Proteomic analysis	QPM inbred lines	Identification of proteins associated with tolerance to combined drought and heat stress.	[28]
Inter-simple sequence repeat (ISSR) and random amplified polymorphic DNA (RAPD) markers.	QPM and non-QPM	Assess the level of genetic variation	[56]
SNP makers	QPM lines	Genetic diversity studies	[28]
SNP-based genetic distance	QPM inbred lines	Heterotic grouping	[20]

The use of molecular markers in QPM breeding programs shorten the selection process during development of improved genotypes, making it more efficient across environments. It is important to note that the newer generation of markers such as SNPs, are being considered to be relatively more efficient and cheaper than older versions (SSR, RAPD). There are numerous service providers who can offer genotyping services at reasonable cost and affordable for researchers in developing countries.

7. QPM Varieties Released in Some Countries around the World

Numerous open pollinated QPM varieties and different type of hybrids have been released in 34 countries worldwide [34]. In addition to these, a double cross QPM hybrid was released in Brazil in 1997 and a QPM hybrid (Zhongdan 9407) was released in China; in India, Shakti-1 was also released in 1998, as well as a hybrid of two QPM introgression lines (Vivek QPM-9) in 2008 [61,62]. In the South African variety list, as maintained by the registrar of plant improvement, 16 QPM hybrids (nine white and seven yellow) and four OPVs (Obatampa SR, QS-King, Qsobo, and Nelson's Choice QPM) have been released and are available for farmers [63].

It is notable that several improved QPM genotypes are available worldwide. However, QPM varieties are mostly grown in Ethiopia and Ghana compared to other African countries. The major concern with the available QPM genotypes is the adoption rate which is still much lower than expected. A lot of efforts therefore need to be invested in ensuring that adoption levels of this nutritious type of maize are elevated.

8. Adoption of QPM and Ways to Enhance Adoption

One of the major challenges with QPM varieties could be the dissemination of the material especially for QPM hybrids into the farmer's fields [5]. Ghana is the dominant country for QPM production in Africa with approximately 70,000 hectares planted with the vast majority of QPM seed of an open pollinated variety (Obatanpa) [5]. According to FAOSTAT [64], only 1% of the total area under maize production in Southern Africa is allocated to QPM production. In 2003, more than 23 countries had released different varieties of QPM genotypes for large scale production of about 3.5 million hectares with Mexico accounting for 2.5 million hectares alone [65].

A participatory rural appraisal (PRA) involving 160 experienced maize farmers selected from four districts of two zones in the central highlands of Ethiopia during 2012 showed that few QPM highland maize cultivars were adopted by farmers. However, an old cultivar (BH660), originally released for the mid-altitude agro-ecology, was widely adopted in most highland areas [50].

Despite the numerous QPM varieties released in several countries and diverse reports showing the importance of producing QPM grain, its adoption has been limited worldwide. This could be mainly due to the minimal dialogue between maize breeders and farmers [66] as the yield of QPM varieties is still predominantly below the production performance of the non-QPM varieties in some cases. A study of the factors affecting adoption of QPM technology in the northern zone of Tanzania showed that education of the household head, farmers' participation in demonstration trials, attendance to field days, and numbers of livestock owned positively influenced the rate of adoption of this technology [67].

Some farmers could be willing to grow QPM varieties, but may not be aware about the existing varieties especially in some rural areas in the world. However, numerous farmers are growing QPM varieties that have been released in various parts of the world. One of the obvious challenges appears to be the limited studies on the adoption of QPM varieties worldwide. The scientific community is, therefore, encouraged to share information on the area cover by QPM production. It is also important to notice that based on the characteristics of QPM varieties, QPM growers need to be trained and informed about the challenges facing QPM production, as well as how to overcome these challenges.

9. Isolation Requirement for QPM Production

QPM grain quality loss occurs when a QPM crop receives pollen from normal endosperm maize because the *op2* allele that confers the QPM trait is recessive [10]. Studies conducted to establish the levels of outcrossing from cross pollination in maize at various distances showed that in seed production, cross pollination levels were around 40% at isolation distances of 2.5 m and 1% at isolation distances of 20 m [68]; Twumasi-Afriyie et al. [69] found that the cross pollination of QPM was not significant at approximately 12 m from the nearest conventional maize plant; Burris [70] reported 1.11% cross pollination at distances of 200 m; and Machida et al. [10] found high outcrossing levels of 63% to 83% in the peripheral areas of the QPM crops within 10 m of yellow normal endosperm maize; Baltazar and Schoper [71] reported no outcrossing at 200 m under very dry and calm conditions. The nutritional value of the QPM trait can be optimally sustained if farmers plant relatively large areas in a square form rather than rectangular fields. In that case, the greater part of the QPM grain will not be outcrossed when grown near a non-QPM variety [10]. The QPM trait is conferred by a recessive gene (*op2op2*) and modifiers. The quality protein grains produced in farmers' fields and recycled as seed for next growing season deteriorate over the years mainly through contamination by pollen from non-QPM varieties grown in surrounding areas when the flowering periods overlap [34].

Opaque-2 is a recessive mutation and endosperm specific which can be easily lost when out-crossed by normal maize. Giving the significant effects of QPM varieties in human and monogastric animal nutrition, it is important to isolate QPM plot from normal maize production, as suggested above. The recommended isolation distance could be from 200–400 m depending on the environment. The isolation could also be based on the planting date before or after the planting of the surrounding normal maize plots (very early planting or very late planting of QPM plot using irrigation system at the beginning or at the end of the season, respectively). The use of early maturing QPM genotypes which can cover the growth cycle within a short period of time (60 to 90 days) could reinforce the isolation in time at farmer level for late planting. By sharing information with farmers, they could come out with relevant method of isolation based on their field experiences.

10. Participatory Plant Breeding and Variety Selection

Several studies show the relevance of participatory plant breeding (PPB) and participatory variety selection (PVS) in the release and faster adoption of varieties, better understanding of farmers' varietal selection criteria, enhanced biodiversity, acceleration of dissemination, increased cost-effectiveness, facilitated farmer learning, and empowerment of farmers with new technology [72–77]. Participatory selection approaches are needed because a low percentage of varieties developed by breeders are eventually adopted and utilized by farmers. This is due, in part, because farmers are left out of the selection process [75]. In Uganda, the adoption of improved varieties by farmers was significantly improved by using PPB and PVS [74].

Participatory variety selection (PVS) conducted in 2012 and 2013 in four villages of Chilga district of Northwest Ethiopia showed that farmers preferred BHQPY-545 because of its high quality protein [77]. This study showed that, in the four villages where the evaluation was done using a participatory approach, farmers were willing to adopt their selected variety ahead of four already-released varieties evaluated. PVS in Southeastern Nigeria over three years involving 275 farmers in the promotion of stem borer resistant maize varieties showed the superiority of two stem borer resistant varieties over the local check with one of them (Ama-TZBR-W) being more preferred by farmers; because of its better overall appeal, the increased number of ears harvested, and almost a four-fold increase in the number of marketable cobs when compared with the local check, especially during the period of high stem borer incidence. The cost-benefit analysis showed a return of N35 for every Naira invested by the farmer on cultivation of that resistant maize variety in the study area [75]. Most often, breeders may discard many crosses and varieties during the selection process because the traits considered undesirable may actually be of interest to farmers [77].

To improve viability of producing QPM genotypes, innovative and sustainable initiatives could be used to create increased demand of the crops through full value chain development. The sustainable food value chain development paradigm recognizes that food insecurity is a symptom of poverty. Financially-resourced households create demand that drives the supply of food. On the supply side, competitive improvements in the food system will reduce food costs [78]. The full value chain development could be used in conjunction with participatory approaches to facilitate wider technology promotion and adoption. The technology adaptation and transfer for processing of QPM maize to shelf-stable food products could be considered.

Widespread adoption and utilization of QPM by smallholder farming communities, who constitute the majority of intended beneficiaries in the developing world, should therefore be encouraged. Adoption of a well thought-out theory of change will be a necessity. Any interventions need to capacitate (with knowledge, attitudes, skills) and influence behavioral change (in actual practices that occur) among the beneficiaries as well as other stakeholders who provide guidance and various services to farmers [79]. Other relevant stakeholders include researchers, governments' agents, policy-makers, private sector (seed companies, millers, feed manufacturers) and NGOs. Stakeholders and beneficiaries could, therefore, be exposed to different technologies in a participatory manner, and the benefits of technologies illustrated through various demonstrations that include feeding trials. Knowledge sharing and innovation platforms could be created to facilitate acquisition of new knowledge that will lead to the adoption of farmer-preferred QPM varieties.

The participatory approaches create efficiency, probably shorten the period of adoption of new varieties, new technology or new practices acceptable at farmer level, but are costly. Farmers can substantially adopt any variety that they have selected based on their preferred criteria from a participative selection process. Researchers should enhance the collaboration with farmers during the selection process of new or improved QPM varieties to facilitate their adoption once released. Full value chain development could be instrumental in increasing visibility, viability, and adoption of QPM materials.

11. Conclusions

QPM could have an impact in areas where maize constitutes a large proportion of the diet, especially as a source of protein, and where children, lactating mothers, and refugees suffer protein deficiency. Even though interdisciplinary research leading to the development of improved varieties is still ongoing, several QPM genotypes have already been released worldwide. QPM production requires an isolation distance range of 200–400 m depending on the environment. Based on the nutritional quality of these varieties, there is a need to raise the adoption rate of QPM worldwide especially in developing countries where a large proportion of maize is produced by small-scale farmers, who use the grain mainly for their own consumption or save the seed for the next growing season. One of the ways of doing so could be the enhancement of the collaboration between researchers and farmers through participatory approaches in the breeding process, which could draw their attention to the existence, availability, nutritional benefits, and production requirement of the improved QPM genotypes.

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