Journal of Biology, Agriculture and Healthcare
ISSN 2224-3208 (Paper) ISSN 2225-093X (Online)
Vol.6, No.15, 2016
www.iiste.org

Review on Quality Protein Maize Breeding for Ethiopia

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Abstract
Maize (Zea mays L.) is a major cereal crops in the World and stable food crops in developing countries. But almost all maize varieties cultivated in the Ethiopia are normal maize varieties, which are deficiency of essential amino acids such as lysine and tryptophan and exposed to malnutrition people feed maize as stable food. Due to this reason substituting the normal maize grown in developing country with QPM would substantially improve the protein status and greatly reduce the malnutrition problems of resource poor people depending on maize as staple food. Quality protein maize (QPM) is a maize variety that possesses significantly higher levels of two essential amino acids, lysine and tryptophan as compared to Normal Maize (NM) varieties, to the presence of the opaque-2 gene in a homozygous recessive state. Maize breeders began transferring opaque-2 genes into local maize varieties , and they enthusiastically rushed the new crop into production .The breeding of QPM involves the manipulation of three distinct genetic systems. The recessive mutant allele of the Opaque-2 gene is the first and central component, the second is comprised of the alleles of endosperm hardness modifier genes and the third is comprised of a distinct set of amino acid modifier genes (termed here "aa-modifiers") which affect the relative levels of lysine and tryptophan content in the grain endosperm. The undesirable characteristics include reduced yield than normal maize, low grain consistence and a farinaceous endosperm that retains water is a major challenge during early development of QPM dissemination. In order to overcome these apparent limitations for large scale use of such mutants, efforts were directed towards identification of alternative mutants that did not carry such disadvantages. The effort, spanning over the last decade, involved collaborative CIMMYT/donor funded projects with large components of flow through funding to enable the full participation of regional NARS. CIMMYT remained the major source of global QPM germplasm and hence QPM development in the region and Ethiopia heavily depended on the large pool of QPM source germplasm available at CIMMYT. Support from DFATD to Ethiopia has continued under the Nutritious Maize for Ethiopia (NuME) project since 2012. Now a day's six QPM varieties are released in Ethiopia for different Agro-ecology of maize production area, But still great effort was expected from maize breeders to convert normal maize to QPM to limit malnutrition in the country.
Keywords: aa-modifiers, malnutrition, Opaque-2 gene, Quality protein maize,

INTRODUCTION

Maize (*Zea mays L.*) is an important cereal crop of the World. It is a member of grass family poaceae and is highly cross pollinated crop. Maize (2n = 2x = 20) is the third most important cereal crop in the world after rice and wheat, and it is believed to have originated in Mexico, and to have been introduced to Ethiopia in the 1600s to 1700s (McCann, 2005).It is cultivated in a wider range of environments than wheat and rice because of its greater adaptability (Koutsika-Sotiriou, 1999).

In Ethiopia, maize grows under a wide range of environmental conditions between 500 to 2400 meters above sea level. The mid- altitude, sub-humid agro-ecology is the most important maize producing environment in Ethiopia (Kebede *etal.,* 1993). This region is considered to be the major maize growing zone in the country. The region lies at an altitudes of between 1000 to 1800 m above sea level and receives a fairly reliable average annual rainfall (1000 to 1500 mm/year), rendering it a region of high potential for maize production. Over half of all Ethiopian farmers grow maize, mostly for subsistence, with 75 % of all maize produced being consumed by the farming household. Ethiopia is already a significant maize producer in Africa, and this role could be further enhanced. Currently, Ethiopia is the fourth largest maize producing country in Africa, and first in the East African region (FAO, 2012).

It is also significant that Ethiopia produces non-genetically modified (GMO) white maize, the preferred type of maize in neighboring markets. But, estimates indicate that the current maize yield could be doubled if farmers adopt higher quality inputs and proven agronomy best practices. At present about 40% of maize planted area make use of improved varieties of seed (Tsedeke, *etal.*, 2015).Maize is mainly grown in the four big regions of the country: Oromia, Amhara, SNNP, and Tigray. Oromia and Amhara contribute to almost eighty percent of the maize produced in 2012 (CSA, 2012*)*.Other regions such as BenishangulGumuz and Gambela also grow maize and have the potential to increase their current production level in the future (ATA, 2012)..

Maize is Ethiopia's leading cereal in terms of production, with 6.2 million tons produced in 2013/2014 by 9.3 million farmers across 2 million hectares of land (ATA, 2014). Over half of all Ethiopian farmers grow maize, mostly for subsistence, with 75 % of all maize produced being consumed by the farming household.

Almost all maize varieties cultivated in the country are normal maize varieties which are devoid of essential amino acids such as lysine and tryptophan. Normal maize varieties grown in Ethiopia cannot sustain normal growth and adequate health of target groups depending on maize as staple food. Because the nutritional profile of maize is poor as it is deficient in essential amino acids such as lysine, tryptophan and methionine due to a relatively higher proportion of prolamines in maize storage proteins which are essentially devoid of lysine and tryptophan.

Millions of smallholder farmers in the major maize producing regions of Ethiopia depend on maize for their daily food throughout the year and they have almost no access to protein sources like meat, eggs and milk for their daily consumption (Dereje *etal.*, 2001). About maize, biochemists had told 90 years ago that maize protein is nutritionally deficient because of the limiting quantities of two essential amino acids lysine and tryptophan (Vasal, 2001).

Therefore, substituting the normal maize grown in Ethiopia with QPM would substantially improve the protein status and greatly reduce the malnutrition problems of resource poor people depending on maize as staple food. Because Quality protein maize (QPM) is a maize variety that possesses significantly higher levels of two essential amino acids, lysine and tryptophan as compared to Normal Maize (NM) varieties. The higher levels of lysine and tryptophan are due to the presence of the opaque-2 gene in a homozygous recessive state which contributes to doubling the biological value of maize (Bressani, 1992). Due to this reason the review of quality protein maize breeding were carried out to understand the role of quality protein maize for those poor people depending on maize as staple food, to understand way to improve protein content of normal maize through breeding program and commercial QPM varieties released in Ethiopia

REVIEW

The nutritional well-being and health of all people are vital prerequisites for the development of societies. Significant advances have been made in genetic enhancement of crop plants for nutritional value. However, malnutrition still remains a widespread problem, and is particularly severe in developing countries with low per capita income. In developing countries, about 32% of preschool children are stunted, and 20% are underweight due to protein malnutrition (Black *et al.*,2008).Globally, nearly 200 million children younger than five years are undernourished for protein, leading to a number of health problems, including stunted growth, weakened resistance to infection and impaired intellectual development. The intricate web of interconnections among nutrition, health, agri-culture, environment, literacy, public policies and countless other factors, impose formidable challenges to the rapid improvement of the nutritional status of eco-nomically deprived sections of the society. However, science and technology have been immensely aiding mankind's continuing efforts to combat poverty, hunger and malnutrition (Prasanna *etal.*, 2001)

Cereals are the only source of nutrition for one-third of the world's population especially in Developing and underdeveloped nations of Sub-Saharan Africa and South-east Asia. The three major cereals, rice, wheat and maize constitute about 85% of total global cereals production amounting to about 200 million tons of protein harvest annually at an average of 10% protein content, out of which a sizeable proportion goes into human consumption (Shewry, 2007). A major concern in case of developing nations is that in most cases, a single cereals crop is the major food staple and as such the nutritional profile of cereal crops assumes great significance. Grain protein content of cereals has a very narrow range with rice (5.8-7.7%), maize (9-11%), barley (8-15%) and wheat (7-22%) as reported by various workers.

Quality Protein Maize

Quality protein maize (QPM) is a maize variety that possesses significantly higher levels of two essential amino acids, lysine and tryptophan as compared to Normal Maize (NM) varieties. The higher levels of lysine and tryptophan are due to the presence of the opaque-2 gene in a homozygous recessive state which contributes to doubling the biological value of maize (Bressani, 1992; Vivek *etal.*, 2008). Breeding for improved protein quality in maize began in the mid-1960s with the discovery of mutants, such as *opaque-2*, that produce enhanced levels of lysine and tryptophan, the two amino acids deficient in maize endosperm proteins. However, adverse pleiotropic effects imposed severe constraints on successful exploitation of these mutants (Krivanek *etal.*, 2007). Interdisciplinary and concerted research efforts led to improve the negative features of the opaque phenotype, and the reincarnation of Quality Protein Maize (QPM). QPM holds superior nutritional and biological value and is essentially interchangeable with normal maize in cultivation and kernel phenotype (Panda *etal., 2010*)

Table 1. Lysine and tryptophan levels as percentages of total protein in whole grain flour of conventional and QPM (o2o2) genotypes**.** Source: (Vivek *etal.,* 2008)

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)

Three Purdue University scientists Edwin T. Mertz, Oliver E. Nelson and Lynn S. Bates-were start searching for maize kernels (actually endosperms) high in lysine since 1963.Using the recently developed automatic amino acid analyzer, they found one mutant maize that had about twice the normal levels of lysine and tryptophan. This new maize produced soft opaque kernels instead of the hard , transparent ones typical of most maize grown in the world, but its composition would make it extraordinarily nutritious by comparison with normal maize (Prasanna *etal*., 2001)

News of this discovery came at a time when protein malnutrition and the protein gap were among the most discussed world problems. It energized nutritionists, plant breeders, and decision makers. In opaque-2 maize they saw for the first time a way to raise the nutritional quality of a vital cereal food. All around the world , maize breeders began transferring opaque-2 genes into local maize varieties and they enthusiastically rushed the new crop into production. Quality Protein maize hybrid programs at CIMMYT were started in 1985, in response to increasing interests in hybrids among the national programs especially in developing countries. The maize QPM breeding program is complex process since it requires the simultaneous manipulation of three genetic systems the opaque-2 gene, the endosperm modifier genes, and the genes that control the lysine content (Krivanek *etal.*,2007).

Quality protein maize Genetics and Breeding strategies

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

O2O2 x *o2o2*

⇩

O2o2

⇩

1/4 O2O2; 1/2 O2o2; 1/4 o2o2

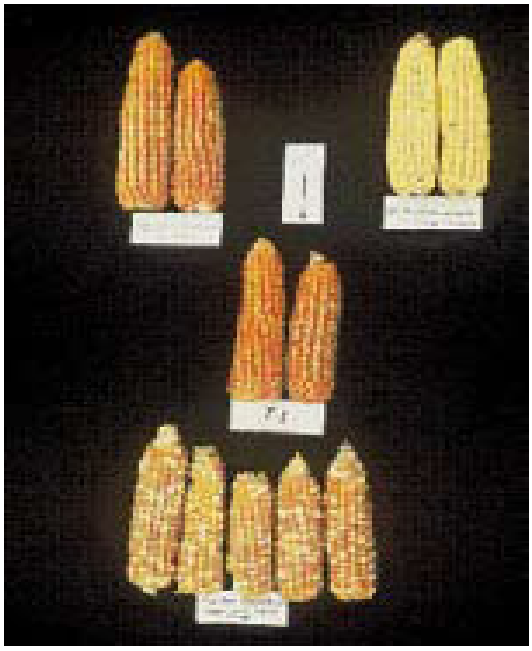

Figure 1. Simple recessive inheritance of the o2 gene. (source;Vivek *etal.,* 2008*)*

The second distinct genetic system managed within a QPM breeding program is comprised of the alleles of endosperm hardness modifier genes (termed here "en-modifiers") which convert the soft/opaque mutant endosperm to a hard/vitreous endosperm with little loss of protein quality. It has been shown that increased levels of gamma zein likely contribute to the recovery of a hard endosperm phenotype as the o2 modified (QPM) grains have approximately double the amount of gamma zein in the endosperm relative to the o2 only mutants (Wallace *etal*., 1990). These en-modifiers along with the o2 mutant allele can be selected for using a rapid and low cost method of selection, whereby light is projected through the vitreous grains or blocked by the opaque grains respectively. Grain endosperm opaqueness is rated on a scale from 1(completely hard/vitreous) to 5 (soft/opaque). All grains with a score of 2-5 are homozygous for the o2 allele, but only grains with score 2-3 have sufficiently modified hard endosperm to be selected as QPM grains. Using this semi-quantitative measure, two genetic loci which affect the modification of the endosperm hardness in o2o2 backgrounds have been mapped to the long arm of chromosome 7 (Lopes *etal.,* 1995) and interestingly one endosperm modifier locus maps near a gamma zein gene gzr1' (Maize Genetics/Genomics Database www.maizegdb.org). Genetic variance accounted for by these two major loci was not calculated in the study but it is likely that other en-modifier loci are also involved in the endosperm hardness modification.

Figure 2. Ears of Pool 25, cycle 0, soft endosperm o2 maize (left) and ears of itsimproved version (cycle 18) (right).Source: (Vivek *etal.,*2008)

Note: Both c0 and c18 have high lysine and tryptophan levels. However, c18 is considered QPM because it also has desirable kernel characteristics.

The third genetic system critical to a QPM breeding program is comprised of a distinct set of amino acid modifier genes (termed here "aa-modifiers") which affect the relative levels of lysine and tryptophan content in the grain endosperm. The lysine levels in normal and QPM maize average 2.0% and 4.0% of total protein in whole grain flour respectively, but range across genetic backgrounds from 1.6 - 2.6% in normal maize and 2.7 - 4.5% in their o2 converted counterparts. Lysine and tryptophan levels are highly correlated (Hernandez and Bates, 1969) and as such quantitative test for either amino acid can be used for analyzing protein quality, although in practice the latter is most often chosen due to lower laboratory costs. Multiple genes have been identified in controlling amino acid content.

At least three gene loci have been implicated in controlling the levels of a protein synthesis factor correlated with lysine levels and these have been mapped to locations on chromosomes 2, 4, and 7. In the same genetic mapping studies free amino acid content (including lysine) was measured using an alternative ninhydrin assay and nine significant loci were identified on chromosomes 1, 2, 3, 4, 5, 7, 8 and 9. As a result of these studies it has become apparent that the simple genetic nature of opaque-2 maize has transformed into a classic polygenic trait in reference to QPM and must be manipulated as such in breeding programs. If lysine or tryptophan levels are not continuously measured during the breeding process the additional gains in protein quality may be lost even though the o2o2genotype is maintained (Vivek *etal*., 2008)

A modification of the second and third strategies is also often employed where by a single backcross generation to the normal endosperm parent is employed to increase the frequency of favorable yield and agronomic alleles, followed by selection within the resulting segregating family. Within each of these methods, successive inbreeding of the material is made in parallel with continual selection on the three QPM genetic systems, line *per se* performance and test-cross performance based on yield, agronomic characteristics and disease resistance. The primary end products are inbred lines used in formation of QPM hybrids and QPM synthetic OPV's. The time required to develop an inbred line with the first two strategies is approximately nine cycles. Nevertheless, if the breeding program has a sufficient pool of elite QPM lines, the first strategy is the simplest as the *o2* allele is fixed within the segregating family and selection is only required on en-modifiers and aa-modifiers and confusion of completely modified *o2o2* genotypes with normal genotypes can be avoided (Krivanek. *etal*.,2007)

Current QPM breeding strategies at CIMMYT focus on pedigree breeding, whereby the best performing inbred lines, complementary in different traits, are crossed to establish new segregating families. New inbred lines are developed from these segregating families in the same process as from the broader based populations.

Three types of crosses provide a choice of breeding strategies: QPM by QPM, QPM by Normal and QPM by Normal Backcross Conversion (of the normal genotype to QPM using at least three backcross generations)(Krivanek *etal*.,2007)

Early QPM Development

The discovery of high-lysine mutations in maizearoused great optimism and considerable interest worldwide, as many believed that it would soon lead to development of nutritionally enhanced cereals. Breeding programmes were initiated in maize to develop in-bred lines and populations using various endospermquality mutants, mainly o2in the initial stages, both o2and fl2genes were used singly or in combination with each other. Later, as some undesirable effects of fl2 mutant were discovered, its use slowed down and was discontinue (Prasanna *et al*., 2001).

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

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

Fortunately, during the process of converting normal maize populations to *o2* versions, partially hard endosperm (i.e. vitreous) or modified grains had been observed by many researchers including breeders at CIMMYT in Mexico. Separation of such grains when encountered began as early as 1969 by Dr. John Lonn-quist (Vasal, 2000). Besides, the first published report highlighting the importance of such grain modification in reducing the negative pleiotropic effects of the *o2* mutation was published in 1969 (Paez *et al.*, 1969).

Selection for hard endosperm modification was rapidly incorporated into *o2* breeding schemes. Occurring at the beginning QPM breeding efforts at CIMMYT focused on conversion of a range of subtropical and tropical lowland adapted, normal endo-sperm populations to *o2* versions through a backcross-recurrent selection procedures, with a focus of accumulating the hard endosperm phenotype, maintaining protein quality and increasing yield and resistance to ear rot (Villegas *et al*, 1992). The improved populations were released for direct use in the field as open pollinated varieties (OPV's), or individual plants were self-pollinated to form inbred lines used in hybrid formation. Similar programs with sustained breeding of QPM also continued at the University of KwaZulu-Natal (previously University of Natal), South Africa and the Crow's Hybrid Seed Company at Milford, Illinois USA (Prasanna *etal.,* 2001).

As a result many cultivars both OPV's and hybrids with improved protein quality were developed for temperate, tropical highland, and for subtropical and tropical lowland growing conditions. The resulting genotypes with elevated lysine and tryptophan content relative to normal maize but without the negative soft endosperm phenol-type were termed by CIMMYT as Quality Protein Maize (QPM). The term QPM now refers to maize homozygous for the *o2* allele, with increased lysine and tryptophan content but without the negative secondary effects of a soft endosperm. QPM looks and performs like normal maize and can be reliably differentiated only through laboratory tests. It should be highlighted that QPM is the product of conventional breeding and no genetic engineering was used during its development.

a

b

Figure 3. QPM (H.E.o2) ears showing a hard, vitreous kernel phenotype comparable to that of normal maize.*a*, Soft, chalky texture of the *o2* kernels without the endosperm modifiers; ***b***, Backlit kernels from normal, soft *o2* and QPM (H.E.*o2*) ears, illustrating the extentof endosperm modification in the QPM kernels.(Source; Prasanna, *etal.*,2001)

Challenges with high lysine mutants

Despite the fact thathigh lysine mutants aroused tremendous interest and enthusiasm for their possible use in developing maize with superior protein quality, but rapidly the negative plieotropic (pleiotropic) effects of such mutations began to be recognized. These undesirable features were limiting factors to its widespread use and adoption (Lauderdale, 2002). Therefore, when even endo-sperm protein mutants such as *o2* and *fl-2* favorably change the amino acid profile of maize grain, they also cause certain undesirable consequences, as is expected of most mutants. The undesirable characteristics include reduced yield than normal maize, low grain consistence and a farinaceous endosperm that retains water (Toro *etal.*, 2003).

These features result in a soft, chalky endosperm that dried slowly making it prone to damage, a thick pericarp, more susceptibility to diseases and pests, higher storage losses and also affects harvest ability. Since the kernel weight is reduced due to less density per unit volume as starch is loosely packed with lot of air spaces, there is corresponding decline in the yield (Singh and Venkatesh, 2006) which can be almost to the tune of 10 percent or above. Especially in developing countries, where farmers are accustomed to hard flint and dent grains, the kernel appearance of such mutants made it less ideal for large scale use and adoption in target areas. The mutations that alter grain protein synthesis cause changes in texture of grains. The early opaque-2 (o2) mutants had reduced levels of α-zeins resulting in small unexpanded protein bodies (Geetha *etal*., 1991), whereas, o15 that reduces -zeins leads to smaller number of protein bodies. Other mutations such as floury-2 *(fl-2), Mucronate (Mc)* and defective endosperm (De B30) result in irregularly shaped protein bodies. In order to overcome these apparent limitations for large scale use of such mutants, efforts were directed towards identification of alternative mutants that did not carry such disadvantages.

This resulted in identification of additional mutants of opaque and floury series, even though none of them eventually could get to farmers fields. This intense the spirit with which high lysine maize research was pursued as the complexity of the coordinate gene action governing endosperm protein profile became more and more evident. Another major setback to opaque-2 mutant research came in 1973 when WHO and UN revised the energy and protein recommendations. The energy was given more priority over protein for defining them as major limiting factors in malnutrition (Lauderdale, 2002). As a result, the interest of researchers got distinctly polarized towards high energy rather than high protein, therefore, a renewed focus towards higher yields to meet energy demands.

Quality protein maize dissemination

A major challenge with QPM is the dissemination of the material into the farmer's field.The dissemination and adoption of QPM is still lagging behind normal endosperm maize especially in regions such as sub-Saharan Africa where it is needed most including Ethiopia (Aman *etal.*,2016). In sub Saharan Africa, total maize area is estimated at 30 million hectares (FAOSTAT, 2012), and only less than 1% (or 200 000 hectares) was estimated to be under QPM. Unfortunately, in the early 1990's the CIMMYT QPM breeding program was discontinued and as such the critical step of promoting this improved material was also severely limited. Since the late 1990's however, the Nippon Foundation of Japan and then later the Canadian International Development Agency (CIDA) have funded the continued improvement and promotion of QPM in several developing countries (Cordova, 2000).

The target countries for large scale cultivation of QPM have been those where maize finds substantial use for human consumption and animal feed. These countries have different levels of development ranging from developed nations like Mexico and Brazil to developing/underdeveloped nations of Africa and Asia. Among humans, women and children have been major targets while as in case of animals, pig and poultry are major targets. In 1977, only four countries grew QPM but in 2003, more than 23 countries have released QPM varieties for large scale cultivation on area over 3.5 million hectares with Mexico alone accounting for about 2.5 million hectares (Sofi *etal.*, 2013)

The Nippon funded project the improvement of quality protein maize in selected developing countries, focused on promotion of QPM in countries, where maize is a staple and where the probability of adoption and impact is high. In sub-Saharan Africa, 17 countries are growing QPM on around 200000 hectares with Ghana alone accounting for about 70000 hectares, Obatampa being the major cultivar. Nippon foundation, CIDA and Rockefeller foundation have been instrumental in promoting the development and dissemination of QPM in Africa (CIMMYT, 2005).In China, a number of high yielding QPM hybrids are under cultivation covering an area of about 1000 hectares. It is expected that by2020, about 30% of maize area in China will be under QPM cultivars (Gill, 2008).

Quality Protein Maize breeding in Ethiopia

Quality protein maize germplasm development in Ethiopia was part of a regional collaborative effort between CIMMYT and a regional network of national agricultural research systems (NARS) in the Association of Strengthening Agricultural Research in Eastern and Central Africa (ASARECA) region involving research, extension and seed production personnel in the Eastern and CentralAfrica (ECA) countries. The effort, spanning over the last decade, involved collaborative CIMMYT/donor funded projects with large components of flow through funding to enable the full participation of regional NARS. CIMMYT remained the major source of global QPM germplasm and hence QPM development in the region and Ethiopia heavily depended on the large pool of QPM source germplasm available at CIMMYT. In particular, the Quality Protein Maize Development (QPMD) project funded by the Canadian International Development Agency (CIDA) has greatly supported QPM germplasm development in four countries in the Horn of Africa (Ethiopia, Kenya,Uganda and Tanzania) since 2003. As part of the project, CIMMYT placed one senior maize breeder in Ethiopia to work with the national program breeders to develop QPM for the region (Worku *etal*., 2012*).*

Research in QPM is of recent history in Ethiopia. The work was started by testing introduced CIMMYT QPM pools and populations in 1980.Alemaya University of Agriculture pioneered in testing these materials. Later, in 1981, two sets of QPM trials were introduced from the same source and evaluated at Melkasa Agricultural Research Center. The set of trials consisting of six QPM hybrids and two OPVs were introduced from Ghana in 1995 in collaboration with SG2000. This trial was evaluated at Bako for two consecutive years (1995-96). BH140 and Beletech were included as local checks in all trials. None of the 19 CIMMYT QPM populations out yielded the local checks at all test locations. This was because they were early maturing and could not compete with the late maturing checks in exploiting the full growing season *(*Leta *etal., 2001)*.

In other study four sets of trials were evaluated at Bako and Awasa Agricultural Research Centers. The results from these trials revealed that some entries produced better grain yield than the normal local check varieties. This showed that there was the possibility of locally developing QPM varieties which could substitute or complement the current normal maize varieties without sacrificing grain yield. From experimental variety trials conducted at some locations, for instance, some QPM entries yielded 95 q/ha an advantage of 20% over the best local check. These trials were, however, tested only for a single season and selected materials were not advanced to multi-location testing (Mosisa *etal.*,1997)

Later on Quality protein maize lines, hybrids and OPVs are introduced annually from international research organizations like CIMMYT and other national agricultural research centers. The four newly introduced CIMMYT QPM hybrids, the Ghanaian hybrid GH132-28 and the open pollinated variety Obatanpa were put under strip tests in the main season of the year 2000 and evaluated on farmers` fields at 21 sites in diverse agro-ecologies in the country for yield and agronomic performance at different locations (Table 2). The test

environments were Alemaya and Ambo (highland), Bako, Pawe, Awasa and Jima(mid-altitude) and Melkasa and Ziway (moisture stress areas). These trials generated useful information on the performance of the varieties under different environments within a very short time *(*Leta *etal., 2001)*

The first released QPM hybrid, however remained unattractive to the majority of Ethiopian farmers especially in the high potential maize growing areas because of its lower yielding potential and susceptibility to major maize diseases (Worku *etal*.,2012). The local check yielded statistically significantly greater mean yield than all QPM entries, Since the QPM hybrids were early maturing and are not expected to perform like the plants of the late maturing check that exploits the full growing season (Aman *etal.*,2016). To solve this problem a number of released and popular CM cultivars (open-pollinated varieties; OPVs, and parental inbred lines of hybrids) were converted to QPM following a backcross breeding procedure described by Vivek *etal*., 2008.

Table 2. Grain yield (q/ha) of QPM hybrids evaluated in strip test at different locations in2000

		Location							
No.	Entry	Bako	Jima	Melkasa**	Alemaya*	Awasa	Ambo	Pawe	Mean
1	P62.XCML150 X CML140	70	76	83	137	7 7	77	70	85
2	CML141 X CML144 X CML176	69	83	96	101	79	66	68	80
3	CML175 X CML176	50	77	104	109	76	47	73	77
4	CML144 X CML159 X CML176	80	91	95	121	83	69	64	86
5	GH132-28	78	81	109	138	88	69	71	91
6	BH540 (Local check)	73	82	103	114	108	87	45	87
	Mean	70	82	93	122	73	59	65	

* On station yield ** One trial fully irrigated, one supplemented (Source Leta *etal*., 2001)

From this experiment the mean yield data combined across locations ranged from77-91 q/ha. The mean grain yield obtained from other hybrids was comparable for or at par withBH540 except the single cross CML175 x CML176 which yielded 10 q less per hectare. The analysis of variance using the AMMI model for grain yield of the QPM hybrids revealed that mean squares of genotypes, environments, and GEI were all significant. The study further indicated that in Ethiopia the QPM hybrids' yield performance was greatly influenced by the environment main effect while the GEI contributed the least to total variation (Demissew, 2014)

Similar trends were observed for other agronomic characters (Table 3). However, clear differences were observed among the QPM hybrids in their reaction to leaf disease. Almost all of them were susceptible to at least one of the three important leaf diseases, viz, common rust, turcicum leaf blight and/or GLS (Leta *etal*.,2001). In contrast in other study most of the QPM hybrids and open variety manifested tolerant to TLB, CR and GLS (Adefris *etal.*, 2015). In the highland areas of Ambo, severity of turcicum leaf blight was as high as 2.5-3.5 ina 1-5 disease scoring scale, where 1 and 5 indicate resistant and susceptible, respectively.

In the mid-altitude areas Bako and Jima, most of the varieties were susceptible to either common rust or GLS. The severity of these diseases was up to3.5-5 on the same scoring scale. It was onlyCML144 x CML159 x CML176 that had the combination of desirable agronomic characters with superior resistance to GLS and other diseases compared toBH540. With all these merits, (CML144 x CML159) x CML176 was proposed for further verification and demonstration to the National Variety Release Committee (NVRC) for possible release in the year 2001.Whereas in Ambo and Awasa the local check was superior to the QPM varieties. However, even if the variety (CML-144 × CML-159) × CML-176 was significantly higher yielding than other tested varieties except GH-132-28 in Bako area (Leta *etal.*,2001)

Table 3. Summary of major agronomic traits of QPM hybrids evaluated in strip testin2000

		Gran yield(q/ha)	Days to silking (no.)	Plant height (cm)	Ear height (cm)	Lodging (%)	Diseases (1-5)*		
No.	Pedigree						Rust	Blight	Gray leaf spot
1	P62.XCML150 X CML140	85	8	210	110	3.6	1.7(1-2.5)	1.52(1-3.5)	1.6(1-4)
2	Obatanpa	111	84	234	125	9.5	1.8(1-3.5)	2(1-3.5)	2.25(1-4)
3	CML141 X CML144 X CML176	80	86	216	108	11.3	1.6(1-3.5)	1.8(1-2.5)	1.2(1-3)
4	CML175 X CML176	70	86	215	104	15	1.9(1-4)	1.6(1-5)	1.0(1-2.5)
5	CML144 X CML159 X CML176	86	87	222	116	11.3	1.3(1-2.5)	1.7(1-3.5)	0.95(1-1.5)
6	GH132-28	91	77	218	105	12.8	1.9(1-5)	1.9(1-3.5)	1.4(1-4)
7	BH540 (Local check)	87	88	218	114	16.4	1.5(1-1.5)	1.37(1-2.5)	1.8(1-4)
	Mean	88	86	218	112	11.4	1.7	1.7	1.5

( ) Indicates the range of scores for disease severity (source Leta *etal.,*2001)

*1 Indicates resistant and 5 Susceptible

Afterward, with support from the QPMD project, a full-fledged QPM development program was initiated for the highland, mid-altitude, and moisture-stressed maize agro-ecologies of Ethiopia, with emphasis on the (1) Screening QPM varieties introduced from elsewhere for adaptation to local conditions (2) Conversion of popular and farmer-preferred CM cultivars into QPM versions. This strategy was aimed at incorporating the opaque2 gene into parental lines of popular Ethiopian hybrids using the backcross breeding method. In the backcross program, parents of popular hybrids such as BH660 (A7033, F7215 and 142-1-e) were used as recurrent parents, while proven CIMMYT QPM lines (CML142, CML159 and CML176) were used as donor parents.(3) 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 (Adefris *etal.*, 2015)

Quality protein maize varieties, their characteristics and adaptation in Ethiopia

Six QPM varieties (four hybrids and two OPVs) have been released for commercial cultivation in different maize agro-ecologies of Ethiopia (Table4). An OPV is a genetically heterogeneous population maintained by open-pollination, which, when reproduced or reconstituted, retains some distinguishing features. 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. Whereas A hybrid is the product (first filial generation: F1) of a cross between two unrelated (genetically dissimilar) parents, one of which is designated as female and the other male (Adefris *etal.*,2015).

Table 4.QPM varieties released in Ethiopia and their agro-ecological adaptations, disease reactions, and agronomic characteristics. Source; (Adefris *etal*., 2015).

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 mid-altitude	220-250	100-120	145	Dark pink	White	Semi-flint	Prolific	80-90	50-60	T	MT	MS
Melkasa-6Q‡	2008	Low moisture stress	165-175	70-75	120	White	White	Semi-flint	Non-prolific	45-55	30-40	_	T	T
BHQPY545	2008	Moist mid-altitude	250-260	120-140	144	50% white & 50% purple	Yellow	Semi-flint	Highly prolific	80-95		T	MT	MT
AMH760Q	2011	Highland	240-290	143	183	White	White	Semi-flint	Prolific	90-120	55-65	T	S	MT
MHQ138	2012	Low moisture stress and moist mid-altitude	200-235	100-120	140	White	White	Semi-flint	Prolific	75-80		T	T	MS
Melkasa-1Q‡	2013	Low moisture stress	140-160	65-70	90	White	Yellow	Flint	Non-prolific	35-45	25-35	_	T	T

* 1 ton = 10 quintals (qt) DM=days to maturity; RC=research center; FF=farmers' field ;T=tolerant; MT=moderately tolerant; MS=moderately susceptible; S=susceptible.

From this experiment the hybrids QPM varieties were showed maximum grain yield than QPM open pollinated variety (Table 4), this indicated that QPM hybrid variety are more preferable than OPV QPM variety while OPV were resistant to GLS,TLB and CLR disease than hybrid variety. The highest grain yield was recorded for BHQPY545 (80-95qt/ha) next to AMH760Q (90-120 qt/ha). The overall performance of QPM released variety in Ethiopia were had good grain yield, based on this point of view farmers can place normal maize variety with QPM variety.

CONCLUSION

Micronutrient malnutrition alone affects more than 2 billion people, mostly in resource-poor families in developing countries. For example, more than 300 million people in India suffer micronutrient deficiencies, and 35% of the world's malnourished children live in that country. Protein-energy malnutrition (PEM) is potentially fatal body-depletion disorder, which is the leading cause of death in children in developing countries (Babu *etal.*, 2013). Ethiopia has one of the highest rates of malnutrition in Sub-Saharan Africa. Study conducted byWorku *etal*.,(2012*)* revealed that the 88% of house hold in Ethiopia, 56% in Uganda,46% in Tanzania and 41% in Kenya were at risk of inadequate lysine in in takes during post-harvest period, due to this reason the study concluded that improving the nutritional quality of the maize grown areas such as those in QPMD target areas could have significant potential in improving the nutritional status, health and wellbeing of these population.

Human beings as well as a number of farm animals are incapable of synthesizing certain amino acids; this fact has stimulated research on improving the levels of some essential amino acids in staple food crops.

While cereals are primarily deficient in lysine (Lys) and tryptophan (Trp), legumes are found to be significantly short of methionine (Met). Consequently, these three essential amino acids have frequently been the targets of manipulation in maize as well as other food crops. In developed countries, the focus is generally on feed quality, as meat consumption provides a sufficient supply of essential amino acids for humans. In contrast, in developing countries where maize is directly consumed as food, both humanitarian and economic interests prevail (Ufaz and Galili, 2008; Atlin *etal*., 2011).

The current rapid expansion of the human population on earth, particularly in the less developed countries, raises the possibility of widespread, serious malnutrition and starvation for many unless agricultural technology can intervene with appropriate answers to these problems. Plant breeders have been charged with developing varieties that will yield larger quantities of improved quality protein. Since the realization that maize having the opaque-2 gene has markedly improved protein quality, much work has been done in many areas of research to apply this discovery as well as to learn more about alternative methods to attain the same goals. QPM is now of major interest to breeders, geneticists, seed producers and the industry, as its large scale production promises to offer significant benefits (Prasanna *etal*.,2001).

Genetic investigation of the opaque-2 mutant and the modifier genes which restore kernelvirtuousness in Quality Protein Maize (QPM) has provided new marker-assisted and transgenic approaches to achieving improved protein quality in maize. In order to shorten the period normally required for development of QPM hybrids through the conventional method of backcrossing, marker-assisted selection (MAS) was the method of choice, as a few molecular markers were already known within the o2 gene and these makers were capable of detecting the o2 gene even in heterozygous state (Babu *etal*.,2005)

Promotion of QPM development were focused developing countries were maize is used as stable food for human consumption and animals especially in African, South America and Asia country. Currently maize scientists feel proud of QPM research at CIMMYT and elsewhere. CIMMYT maize staff has gained confidence and strength and are aspiring to do more to spread the benefits of this maize to more developing countries (Vasal, 2001).QPM varieties have been released in more than 23 developing countries for large scale cultivation on the area over 2.5 million ha (Akande and Lamidi, 2006; Sofi *etal*., 2009). QPM is primarily developed for tropical and sub-tropical regions, but it could also have many advantages in other parts of the world for animal feed and also in human nutrition.

In particular, the Quality Protein Maize Development (QPMD) project funded by the Canadian International Development Agency (CIDA) has greatly supported QPM germplasm development in four countries in the Horn of Africa (Ethiopia, Kenya, Uganda and Tanzania) since 2003.With technical and material support from CIMMYT and other organizations such as SG2000, significant efforts have been made to develop, release, and disseminate QPM varieties in developing countries where maize is the dominant dietary source of energy and protein, to address the issues of protein under nutrition. The support from Quality Protein Maize Development (QPMD) project funded by Canada's Department of Foreign Affairs, Trade and Development (DFATD) to Ethiopia has continued under the Nutritious Maize for Ethiopia (NuME) project since 2012(Adefris*etal*.,2015)

The first released QPM variety in Ethiopia, BHQP542, had the pedigree CML144/CML159// CML176, which left unattractive to farmers since it is low yielder and susceptible to common maize disease. But currently six QPM varieties were officially released in Ethiopia until 2014, which are yield competent with normal maize. Since large population was used maize as stable food in developing counties, especially in Ethiopia great effort was expected from maize breeders to convert normal maize to QPM to limit malnutrition in the country.

Future Prospects

Understanding the malnutrition problem farmers used maize as stable food in developing county like Ethiopia, and using effective application to improve nutritional content of maize is a challenging endeavor for breeder. Substitution of normal maize with QPM is substantially could improve essential amino acid content problem associated with maize crop and Enriching crop plants in essential amino acids has both economical and humanitarian interest.

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