QUALITY PROTEIN MAIZE (QPM)

A Guide to the Technology and Its Promotion in Ethiopia

CIMMYT

Adefris Teklewold, Dagne Wegary, Abraham Tadesse, Birhanu Tadesse, Kassahun Bantte, Dennis Friesen and B.M. Prasanna

CIMMYT – the International Maize and Wheat Improvement Center – is the global leader in publicly-funded maize and wheat research-for-development. Headquartered near Mexico City, CIMMYT works with hundreds of partners worldwide to sustainably increase the productivity of maize and wheat cropping systems, thus improving global food security and reducing poverty. CIMMYT is a member of the CGIAR Consortium and leads the CGIAR Research Programs on MAIZE and WHEAT. The Center receives support from national governments, foundations, development banks and other public and private agencies.

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Abstract: This guide book introduces the nutritional benefits of QPM over conventional maize varieties and presents a brief overview of its historical development. It also provides information on QPM varieties available for commercial production in different agro-ecologies of Ethiopia and the agronomic management practices required for seed and grain production. It further presents a general guide on how to establish field demonstration plots and conduct field days on QPM varieties. It is meant to serve as a reference for extension experts, farmers and other stakeholders who are involved in the production, demonstration, and extension of QPM in Ethiopia. To some extent, it would be a useful source of information to health professionals who are involved in health extension, and to students and teachers in agricultural training centers such as Agricultural, Technical and Vocational Educational Training (ATVET) colleges.

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Kassahun Bantte, Dennis Friesen and B.M. Prasanna

Nutritious Maize for Ethiopia (NuME) Project
CIMMYT, Addis Ababa, Ethiopia

CIMMYT MR
International Maize and Wheat Improvement Center

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Definitions

Alleles: Alternative forms of the same gene, located at the same locus in a chromosome.

Amino acids: The building blocks from which proteins are constructed. Amino acids are classified either as essential or non-essential.

Backcross: A cross between a hybrid (F_1) and one of its parents.

Conventional maize: A term used interchangeably to describe maize that is not QPM.

Dominant allele: An allele that express itself in the heterozygous form.

Donor parent: In backcross breeding; the parent from which one or more genes are transferred to the recurrent parent.

Dough stage: The stage of maize/cereal grain development at which the kernel's milky inner fluid changes to a "doughy" consistency as starch accumulation continues in the endosperm. In maize this usually happens about 24 to 28 days after silking.

***Edir*:** A traditional "burial society" in Ethiopia to which members make monthly contributions and receive a payment to help cover funeral expenses in return. Nearly every modern Ethiopian is thought to be a member of at least one *edir*, either a neighbourhood association, one based at work, or operating along age or gender lines.

Essential amino acid: An amino acid that cannot be synthesized by the organism being considered, and therefore must be supplied in its diet; whereas non-essential amino acids can be produced from other amino acids and substances in the diet and metabolism.

F_1 (1st filial generation): Progeny obtained by crossing two different parents or the first generation from a cross.

F_2 (2nd filial generation): Progeny obtained by self-fertilization of or crossing between the same F_1 individuals or F_1 individuals of the same population

Genotype: The genetic constitution of an individual organism.

Germplasm: The sum total of hereditary material or genes present in a species.

Githeri: A mixture of boiled maize kernels and beans in a ratio of 2:1.

Gotera (Amharic): A granary made by weaving elongated thin shrub stems or split bamboo sticks plastered with mud and cow dung, usually cylindrical in shape, flat or conical at the base and covered with a conical thatched roof.

Grain/endosperm modification: The extent to which the mutant maize endosperm of the soft (opaque) phenotype carrying the *o2* gene is converted through breeding selection to the hard/vitreous phenotype similar to that of conventional maize.

Height-for-age: The age that corresponds to the child's height when plotted at the 50th percentile on a growth chart.

Heterozygous: An individual having dissimilar alleles of a gene.

Homozygous: An individual having two or more identical alleles of a gene.

Hybrid maize: Maize varieties or cultivars created by crossing two different inbred parental lines (to form a single-cross hybrid) or one inbred line with a single-cross parent (to form a three-way cross hybrid). Other types of hybrids include double-cross hybrids (formed by crossing two different single-cross parents) and top-cross hybrids (formed by crossing an OPV to a single-cross hybrid. Parents of hybrids are chosen on the basis of desired characteristics to combine into a hybrid.

Injera: A leavened bread made from fermented dough.

Lysine: A basic amino acid that is a constituent of most proteins. It is an essential nutrient in the diet of vertebrates.

Monogastric animal: An animal with a simple single-chambered stomach, as compared to ruminant animals such as cows, goats, or sheep, which have a complex four-chambered stomach. Animals with a monogastric digestive tract are less efficient than ruminants in extracting energy from cellulose digestion.

Nutrition: The process of providing or obtaining the food necessary for health.

***opaque2* (*o2*) gene:** A recessive gene in maize responsible for increased lysine and tryptophan contents in the endosperm protein.

Open pollination: Pollination which occurs freely and naturally without restriction.

Open-pollinated variety (OPV): An assemblage of cultivated maize plants distinguished by uniform morphological, physiological, cytological, chemical or other characteristics which, when reproduced or reconstituted, retain its distinguishing features.

Phenotype: The set of observable characteristics of an individual resulting from the interaction of the genotype with the environment.

Protein: Any of a class of nitrogenous organic compounds which have large molecules composed of one or more long chains of amino acids and are an essential part of all living organisms.

Quality protein maize (QPM): The term QPM refers to maize genotypes having the *opaque2* (*o2*) gene and, consequently, generally higher lysine and tryptophan content as compared to conventional maize genotypes, as well as a vitreous endosperm similar to conventional maize to ensure acceptable ear characteristics.

Recessive: An allele of a gene whose action is hidden by the presence of a dominant allele of the same gene.

Recurrent parent: The parent in backcross breeding to which one or more genes from the donor parent are transferred.

Tryptophan: An amino acid that is a constituent of most proteins. It is an essential nutrient in the diet of vertebrates.

Ugali: A stiff, unfermented porridge, prepared by gradually adding maize flour to boiling water and stirring continuously until cooked.

Weight-for-age: An index of the adequacy of the child's nutrition to support growth. Standard weight-for-age is the 50th percentile on a growth chart.

List of abbreviations

ATA	Agricultural Transformation Agency
ATVET	Agricultural, Technical and Vocational Educational Training Colleges
BoA	Bureau of Agriculture
CIDA	Canadian International Development Agency
CIMMYT	International Maize and Wheat Improvement Center
DFATD	Department of Foreign Affairs, Trade and Development, Canada
EIAR	Ethiopian Institute of Agricultural Research
m.a.s.l.	Meters above sea level
MoA	Ministry of Agriculture
NuME	Nutritious Maize for Ethiopia
OPV	Open-pollinated variety
QPM	Quality protein maize
QPMD	Quality Protein Maize Development Project
SG 2000	Sasakawa Global 2000
SNNPR	Southern Nations, Nationalities and Peoples Region
WHO	World Health Organization

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1. Introduction

Maize is first in terms of total production and second in area sown of all cereal crops produced in Ethiopia. Most people in the Ethiopian maize belt rely on maize as a dietary carbohydrate source. The maize varieties presently grown by farmers, hereafter referred to as conventional maize (CM) varieties, are deficient in two essential amino acids, lysine and tryptophan. Essential amino acids are not synthesized through the metabolic processes of monogastric animals such as human beings and must be present in the food consumed by these animals. To overcome this problem, scientists 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 produce a vitreous endosperm resembling that of CM, maize that contains approximately double the amount of lysine and tryptophan has been named as “quality protein maize” (QPM). QPM is a cheap source of protein, given that farmers can grow, manage, harvest, and consume it in the same way they do CM varieties.

Amino acids are the building blocks of proteins. **Lysine** and **tryptophan** are among the **essential amino acids**, i.e., amino acids that cannot be synthesized de novo (from scratch) by the organism and therefore must be supplied in its diet.

Failure to obtain these essential amino acids from the daily diet results in protein deficiency and may be 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.

An important factor that determines protein quality is how closely the ratio of essential amino acids present in a particular food item matches the human requirement. Meat, eggs, milk, and legumes are known to be good sources of essential amino acids. But animal proteins are not affordable for a large segment of small-scale farmers.

1.1 Purpose of this guidebook

This guidebook is meant to serve as a reference to agricultural education institutions, agricultural and health extension experts, farmers, and other stakeholders involved in the production and promotion of QPM in Ethiopia. It introduces the nutritional benefits of QPM over CM and provides a brief overview of its historical development. It gives information on QPM varieties that are available for commercial production in the different agro-ecologies of Ethiopia, together with the agronomic management practices required for grain and seed production. It also gives general guidelines for establishing field demonstration plots and conducting field days for the promotion of QPM varieties.

2. What is QPM?

The term QPM refers to maize genotypes whose lysine and tryptophan levels in the endosperm of the kernels are about twice higher than in CM varieties. Lysine levels in conventional and QPM maize average 2.0% and 4.0% of total protein in whole grain flour, respectively. These levels can vary across genetic backgrounds with ranges of 1.6-2.6% in CM and 2.7-4.5% in converted QPM counterparts (Table 1). Despite the nutritional differences, QPM varieties look and perform like CM varieties and one cannot visually distinguish between the two by the physical appearance of the plants or their ears and grains alone. Rather, biochemical analysis is required to determine the lysine and tryptophan content of the seed and confirm whether or not it is QPM.

Table 1. Lysine and tryptophan levels as percentages of total protein in whole grain flour of conventional and QPM (*o2o2*) genotypes.

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)

Source: Vivek et al. (2008)

Remember!

- The total **quantity of kernel protein** content in both QPM and CM is usually the same.
- It is only the **quantity (percentage share) of the two essential amino acids in the endosperm protein** that is enhanced in QPM.
- Therefore, the nutritional advantage of QPM is due to the increase in **protein quality or amino acid balance, but not to the increase in protein quantity**.

2.1 Genetic background

Understanding the genetic background of QPM is important for QPM breeding, seed maintenance, and production of grain with acceptable lysine and tryptophan content. QPM owes its origin primarily to a naturally occurring mutant, called *opaque2* (*o2*). In QPM, this recessive allele has to be present in a homozygous state (*o2o2*), unlike conventional maize, which has a dominant allele at the same locus, usually in a homozygous (*O2O2*) state. The *o2* gene also enhances the levels of the two essential amino acids in the endosperm. Varieties derived from this original variant/mutant have been used throughout the course of QPM development. Scientists employed conventional breeding approaches to incorporate the *opaque2* gene into a CM background.

QPM development involves manipulating three distinct genetic systems (Krivanek et al., 2007):

a) The simple 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 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 (Figure 1). However, the presence of the *opque2* allele in the recessive condition (*o2o2*) alone does not ensure high lysine and tryptophan levels.

The second essential genetic system involves a set of genes that enhance the levels of lysine and tryptophan in 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. 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.

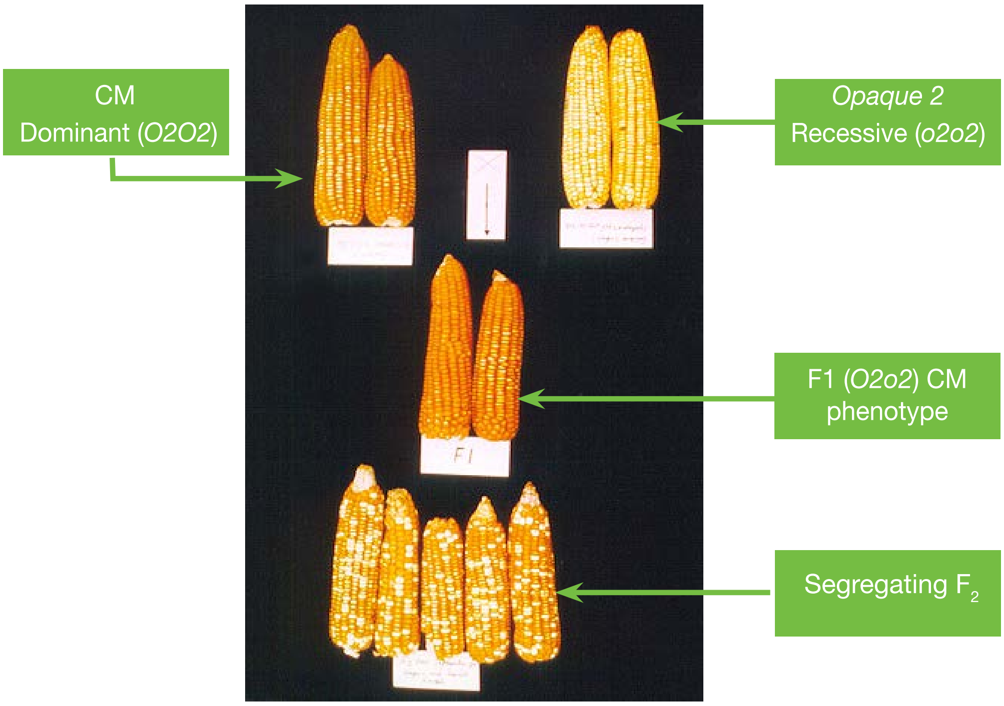


Figure 1. Simple recessive inheritance of the *o2* gene. Source: vivek et al. (2008)

The *o2o2* gene and the modifiers/enhancers of lysine and tryptophan are, by themselves, not sufficient to develop agronomically acceptable maize with high lysine and tryptophan. Due to a genetic phenomenon in which one gene controls more than one trait, the presence of the *o2o2* gene makes the maize endosperm soft and opaque, often making the kernels susceptible to cracking, ear rots, and weevils (Figure 2C). The opaqueness of the kernel can be clearly viewed on a light table (Figure 3). Therefore, breeding maize for high lysine and tryptophan content requires selection for hard kernel texture or vitreousness controlled by modifier genes with a distinct genetic system. The modifier genes convert the soft/opaque endosperm to a vitreous phenotype similar to that of CM.

Figure 2. (A) Normal endosperm flint type maize; (B) normal endosperm dent type maize; (C) *opaque2* maize; and (D) QPM. Source: Krivanek et al. (2007)

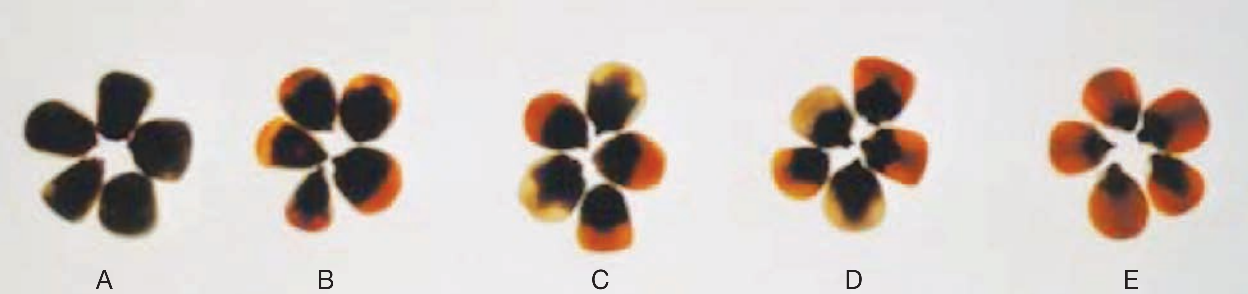


Figure 3. Varying degrees of opaqueness indicate varying levels of endosperm modification: A = opaque; B = 25% modified; C = 50% modified; D = 75% modified; and E = 100% modified. Source: Kassahun and Prasanna (2004)

2.2 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. The salient events of this discovery (Prasanna et al., 2001; Vasal, 2000) 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*).
- 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.
- Breeding programs worldwide 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. These negative effects included:
    - 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 (Vasal, 2000); 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.
- 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.

The resulting genotypes developed by CIMMYT's breeding program were termed Quality Protein Maize (QPM). QPM germplasm is characterized by having higher lysine and tryptophan content than CM, as well as normal vitreous endosperm, reduced susceptibility to post-harvest insect pests and diseases such as ear rots, as compared to their *o2* predecessors, and its yield is comparable to or higher than that of CM grown by farmers. QPM looks and performs like conventional maize and can be reliably differentiated only through laboratory tests. Several QPM populations and pools possessing different ecological adaptation, maturity, grain color, and texture were developed (Prasanna et al., 2001). 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.

Current QPM breeding strategies at CIMMYT 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. 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. Significant strides have also been made with regard to molecular marker-assisted selection (MAS) for generating QPM versions of elite inbred lines. Microsatellite markers located within the *o2* gene made it possible to accelerate the pace of QPM conversion programs through marker-assisted selection (MAS). Recent technological 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 (Babu and Prasanna, 2014).

2.3 Nutritional benefits of QPM

The basic source of QPM's nutritional benefits is the *opaque2* mutation. The higher lysine and tryptophan contents of QPM varieties, compared to CM, provide a more balanced protein for humans and other monogastric animals. There is an overwhelming amount of data demonstrating the nutritional superiority of QPM over CM. The nutritional benefits, especially for people who depend on maize for their energy, protein, and other nutrients, are sufficient to justify its widescale production and promotion.

Numerous QPM feeding trials have been undertaken in areas where participants, most often children, are undernourished. Graham et al. (1990) reported that 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. Combined analysis of various experiments carried out independently in different countries (Gunaratna et al., 2010) indicated that children consuming QPM instead of CM had a 12% weight increase. Meta-analysis of nine experiments, as indicated in Figure 4, provided strong inferences about the nutritional benefits of QPM; seven of these experiments showed that consuming QPM increased child's weight as compared to CM. Gunaratna et al. (2010) also showed a 9% increase in the growth rate of children who received QPM food over those who ate CM. Except in one case, where consuming CM and QPM was statistically not significant in terms of rate of height increase, the other experiments considered in the meta-analysis proved the superiority of QPM over CM in terms of increase in the rate of growth or height of infants and young children (Figure 5). As in the case of infants and children, QPM had equally beneficial effects on adults (Bressani, 1990). Overall, these studies concluded that consuming QPM improves growth rates and nitrogen metabolism, suggesting that it may be as efficacious as consuming casein, the milk protein. Due to the significantly enhanced levels of tryptophan and lysine it contains, QPM also reduces by half the amount of maize that needs to be consumed to get the same amount of biologically usable protein from a maize diet.

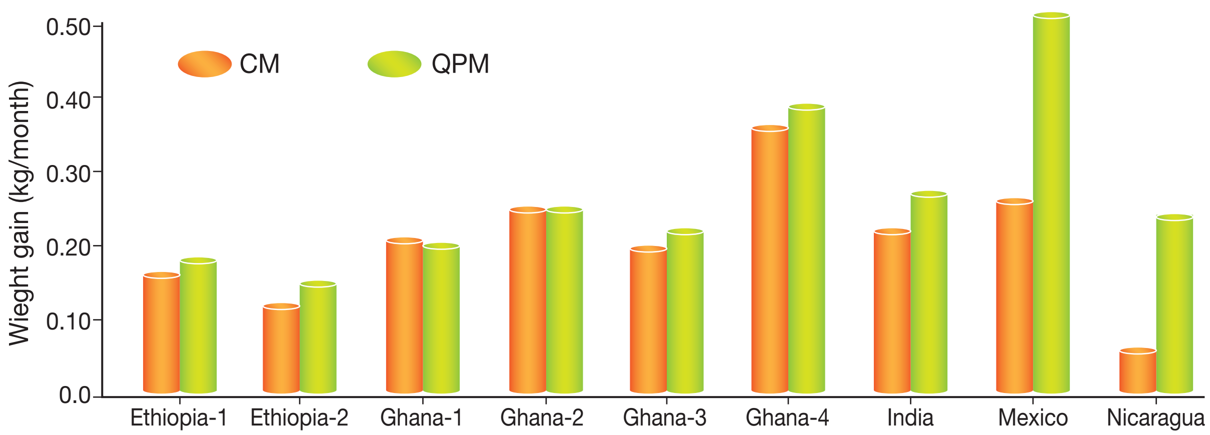


Figure 4. Rate of increase in weight (kg/month) among children receiving conventional maize (CM) versus QPM. Source: modified from Gunaratna et al. (2010)

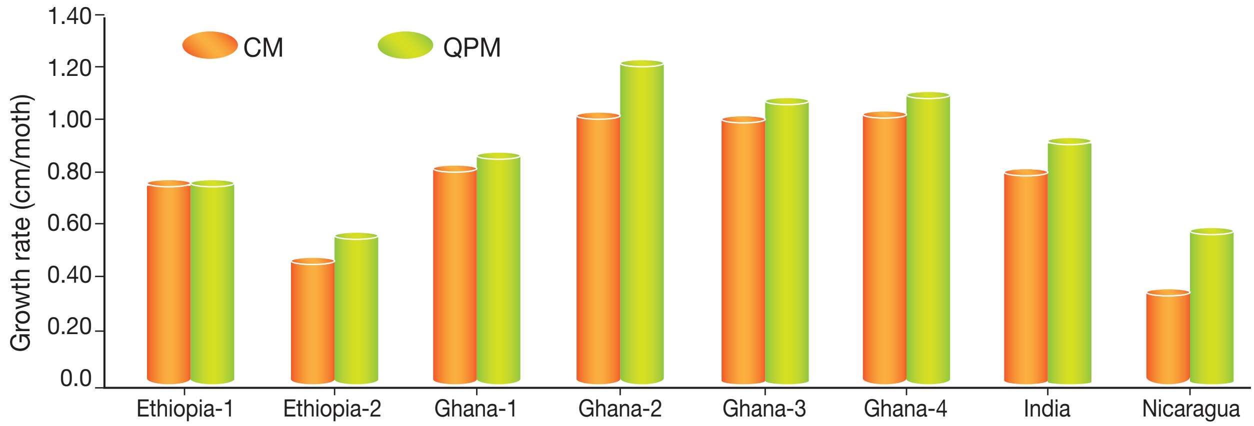


Figure 5. Rate of increase in height (cm/month) among children receiving QPM versus conventional maize (CM). Source: modified from Gunaratna et al. (2010)

Besides doubling the biologically usable protein in a maize diet, QPM also confers the following nutritional benefits: 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 (Bressani, 1992; Graham et al., 1990).

A study conducted by Akalu et al. (2010) in Ethiopia, especially in Sibu Sire Woreda and East Wollega where maize is a dominant crop, demonstrated the positive effect of QPM on both the height and weight of children aged 7 to 56 months. Children consuming CM showed a decrease in both height-for-age and weight-for-age over time, while children fed QPM did not show significant change in height-for-age but their weight-for-age increased marginally.

Based on information collected from a focus group discussion in Sibu Sire Woreda, traditional foods prepared with QPM were appreciated by the farmers for their taste and cooking qualities. Farmers preferred *injera* made from QPM over CM *injera* due to its softness and longer shelf life. 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 longer time after consuming QPM-based food (Akalu et al., 2010). 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, *githeri* in Kenya, and *injera* in Ethiopia.

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 (De Groote et al., 2014).

The nutritional and biological superiority of QPM has also been amply demonstrated in model systems such as rats and pigs. The superior quality of QPM protein was first demonstrated in feeding trials with rats (Mertz et al., 1965). Growth in rats that were fed a diet of 90% QPM (97 g) increased more than three-fold (Figure 6) over the growth of rats fed CM (27 g). The nutritional advantage of QPM over CM was most extensively demonstrated in pigs (Maner, 1975). Generally, at suboptimal protein levels, feeding pigs with QPM instead of an equal amount of CM resulted in significant growth increase. Some studies indicated that pigs fed a diet of QPM alone, except for vitamins and minerals,

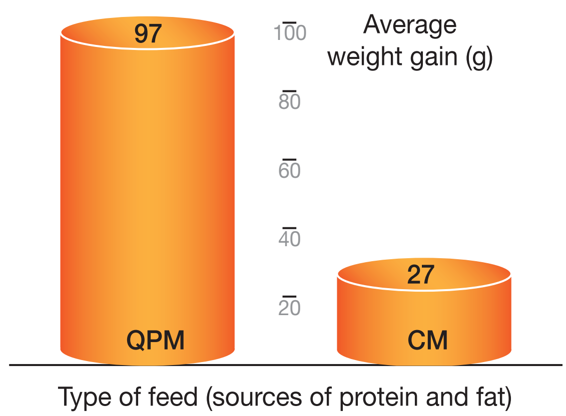


Figure 6. Average weight gain (g) of rats fed on QPM and conventional maize (CM) for 28 days. Source: modified from Mertz et al. (1965)

grew twice as fast as those fed CM (Osei et al., 1995; Vivek et al., 2008). A series of experiments on the nutritional value of QPM in poultry feed (broilers and laying hens) and pigs at the Chinese Academy of Agricultural Sciences (CAAS) proved the superiority of QPM over CM in terms of amino acid balance and nutrient composition, by improving the growth and performance of various animals. Diets incorporating QPM are also more economical, as they can lead to progressive reductions in the use of fishmeal and synthetic lysine additives (Qi et al., 2004).

2.4 QPM germplasm development in Ethiopia

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 undernutrition. The Quality Protein Maize Development (QPMD) project funded by Canada's Department of Foreign Affairs, Trade and Development (DFATD) (formerly the Canadian International Development Agency, CIDA), supported QPM germplasm development and dissemination in four eastern African countries, including Ethiopia, during 2003-2010. The support from DFATD-Canada to Ethiopia has continued under the Nutritious Maize for Ethiopia (NuME) project since 2012.

The QPM development program in Ethiopia was launched in 1994 with the evaluation of open-pollinated varieties (OPVs) and pools introduced from CIMMYT. The main objective of the program was fast-tracking the release of best-bet QPM varieties developed in different CIMMYT maize breeding hubs and elsewhere in the world. It was through this process that the first commercial QPM variety, BHQP542, was identified and released for commercial cultivation in the mid-altitude areas of Ethiopia in 2001. Subsequently, 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 following:

1. *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 from CIMMYT breeding programs in Mexico and other regions. Introduced varieties that showed better or comparable performance to the standard checks, with respect to grain yield, other agronomic traits, and reaction to major diseases were proposed for commercial release.
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. F_1 crosses were made between donor and recurrent parents to transfer the *o2* gene from the donor to the recurrent parents. In the following season, F_1 seeds were advanced to F_2 by selfing the F_1 plants to allow the expression of the target recessive gene. Using a light table, only F_2 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 F_2 generation, where selection for endosperm modification and monitoring the level of tryptophan were carried out on a regular basis.
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. In addition, the pedigree method of inbred line development was used to develop inbred lines, i.e., through repeated selfing of the F_1 (obtained by crossing popular QPM parental lines) for 6-7 generations to select QPM inbred lines from the segregating progenies. After each selfing, kernels were selected for endosperm modification using the light table, followed by tryptophan analysis to identify promising QPM versions of the conventional inbred lines.

With these three strategies, the EIAR National Maize Research Program, in close partnership with CIMMYT, developed and released six QPM varieties until 2014 for the three maize agro-ecologies of Ethiopia. A detailed description of the characteristics and adaptation of these varieties is presented in the next section.

3. QPM varieties, their characteristics and adaptation

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

3.1 Open-pollinated varieties (OPVs)

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. Compared to hybrids (discussed in Section 3.2), OPVs have the following advantages:

- They are relatively easy to develop.
- The seed is simple and inexpensive to produce (it does not have distinct male and female parents and as a result there is no need for detasseling).
- Farmers can save their own seeds for replanting in the following season, thus reducing their dependence on external seed sources (although it is recommended that farmers purchase fresh seed every 3-4 seasons).Seed can easily be transferred from farmer to farmer.

However, OPVs also have some distinct disadvantages, as compared to hybrids:

- They produce relatively lower yields and are not as uniform as hybrids.
- They are not suitable for mechanized harvesting as compared to hybrids.

By 2014, the EIAR National Maize Research Program had released two improved QPM OPVs for commercial cultivation, 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

Table 2. QPM varieties 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 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	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 mid-altitude	200-235	100-120	140	White	White	Semi-flint	Prolific	75-80	55-65	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. Source: Ethiopian National Maize Research Program, EIAR

All varieties except those followed by ‡ are hybrids.

recycled with little or no yield penalty for a few (optimally three) years. However, it should be noted that small plots of QPM OPVs that are surrounded by CM fields are easily contaminated and hence will not maintain the required protein quality. The biological reason for this is presented in detail in Section 4.

i. **Melkassa 6Q:** This OPV was released in 2008 for commercial production in moisture-stressed areas of the country. Its yield potential is 4.5 to 5.5 tons per hectare (t/ha) under research management and 3.0 to 4.0 t/ha under farmers' conditions. On average it takes 120 days to attain grain maturity. This variety is popular in the Central Rift Valley areas of the Oromia and Southern Nations, Nationalities and Peoples (SNNP), Somali regions, and in some parts of Tigray due to its tolerance to low moisture stress during flowering. Seed of this variety is currently being commercially produced by different public and private seed companies and farmers' cooperative unions.

ii. **Melkassa 1Q**: This is a QPM version of Melkassa 1 (a variety that is well known 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 only 90 days. Both this variety and its conventional counterpart are not recommended for relatively high potential maize production areas because of their lower yield compared to other varieties. The yield potential of this variety is 3.5 to 4.5 t/ha on the research station and 3.0 to 4.0 t/ha under farmers' conditions. Farmers who grow this variety should be aware that it is exposed to bird and wild animal attack because of its early maturity and short stature.

3.2 Hybrid QPM varieties

A hybrid is the product (first filial generation: F_1) of a cross between two unrelated (genetically dissimilar) parents, one of which is designated as female and the other male. When the hybrid is formed by crossing two different inbred parental lines, it is a single-cross hybrid. A cross of one inbred line with a single-cross hybrid parent forms a three-way cross hybrid. Other types of hybrids include double-cross hybrids (formed by crossing two different single-cross parents) and top-cross hybrids (formed by crossing an OPV to a single-cross hybrid). BHQPY545 is an example of a single-cross hybrid obtained by crossing two QPM inbred lines: CML161 (the female or "seed" parent) and CML165 (the male parent). Examples of three-way cross hybrids are BHQP542, MHQ138, and AMH760Q.

Advantages of these hybrids include:

- They produce higher grain yields compared to OPVs.
- They have more uniform characteristics (particularly single-cross hybrids), making them more suitable for mechanization.

Figure 7. Performance and appearance of Melkassa 6Q under field conditions.

Hybrids also have some constraints:

- They are more expensive to develop.
- The price of hybrid seed is higher compared to that of OPVs.
- Farmers must purchase fresh F_1 seed every year as use of F_2 results in a yield reduction of as much as 30% compared to F_1 seed.

Some important aspects of the QPM hybrid varieties released in Ethiopia are presented below.

i. **BHQP542 (Gabissa):** This QPM hybrid was released in Ethiopia in 2001 and is adapted to the country's mid-altitude, sub-humid maize agro-ecologies (1000-1800 m.a.s.l.). It has comparable grain yield and shares the same adaptation zones with BH540. It is a three-way cross hybrid involving three QPM inbred parents, all developed by CIMMYT. 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 such as 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, such as Hadiya and Baduwacho in SNNPR, and Illuababora in Oromia.

ii. **BHQPY545 (*Kello*):** This yellow kernel single-cross QPM hybrid was released in 2008 for commercial cultivation in low- and mid-altitude sub-humid maize agro-ecologies. It is derived from two CIMMYT QPM inbred lines, CML161 and CML165, and has been released in several countries globally where it enjoys wide popularity. In addition to its nutritional advantage, this variety is high yielding, lodging resistant, and early maturing. Under good management, this hybrid usually bears two or more ears per plant. 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. Some farmers have managed to produce up to 9.8 t/ha of grain in farmer-managed demonstration plots in Gobu Seyo district in East Wollega. Although consumers generally prefer maize with white kernels, demand for BHQPY545 is expected to increase for the following reasons:

- Increased awareness in the community of the nutritional advantage of the variety, particularly for children, as well as pregnant and lactating women. Another nutritional factor associated with yellow kernel color is elevated provitamin A content.
- Demand for yellow maize, such as BHQPY545, for making corn flakes. In recent years, farmers in Bako Tibe, Illu Gelan, Gobu Seyo, and Sibu Sire districts who cultivate this variety have received premium farm-gate prices from the FAFA food processing factory.
- Demand from the country's flourishing poultry industry for BHQPY545 grain because of its yellow color (to enhance egg yolk color) and protein quality (to supplement protein in rations).
- Its suitability, both in taste and prolificacy, for green ear consumption.

Figure 8. Plant (left photo) and ear (right photo) morphology of BHQP542.

This variety is low to moderately affected by ear rot due to open ear tips under conditions of high fertility. To reduce the incidence of ear rot, growers are advised to apply one of the following strategies:

- Avoid growing this variety in areas where ear rot is prevalent.
- Produce the variety for the green ear market as it is prolific under optimum management conditions.
- Delay planting the variety so that will mature late in the season when rainfall is subsiding or has ended, since ear rots are favored by excessive moisture penetrating the ear.
- Grow the variety during the off-season under irrigation in areas to which it is adapted, thus avoiding excessive moisture as the crop matures.

iii. **AMH760Q *(Webi*)**: AMH760Q, released in 2010, is a three-way cross hybrid adapted to the highland agro-ecologies of Ethiopia (1800 to 2600 m.a.s.l.). *Webi* was produced by a program aimed at converting the parental lines of BH660 into QPM through the backcross breeding method and 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.

Figure 9. Plant (top photo) and ear (bottom photo) morphology of BHQPY545.

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

- *Webi* is susceptible to turcicum leaf blight (TLB). Therefore, farmers in highland areas where TLB is a serious problem are advised to grow other QPM varieties with tolerance to the disease.
- *Webi* has mixed purple and white (50:50) tassels as a varietal characteristic, in contrast to BH660 which is uniformly purple. This mixed tassel color does not indicate seed contamination and has absolutely no effect on grain yield. However, if the proportion of purple and white tassels in *Webi* deviates significantly from 50:50, it could be due to contamination.

Figure 10. Plant (top photo) and ear (bottom photo) morphology of AMH760Q (*Webi*). Please note the mixed tassel color that is characteristic of *Webi.*

iv. **MHQ138:** This three-way cross QPM hybrid was developed for moisture-stressed areas of the country. It is also well-adapted to areas with higher rainfall such as the moist mid-altitude agro-ecologies (e.g., around Bako). This variety has the same female parent as BHQP542 (CML144/CML159), but its male parent is derived from POOL15Q. Consequently, 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. It has shown higher yield potential in on-farm demonstration plots in the vicinity of Bako than when it is grown in moisture-stressed areas. Therefore, due to its earliness, this hybrid could be used as an alternative QPM variety in high potential transitional midland areas.

Figure 11. Plant morphology of MHQ138.

4. QPM variety maintenance

The production and maintenance of QPM seed do not differ from those of CM seed. The same strict standards in terms of land preparation, isolation distance/time, roguing, field management and inspection, detasselling, post-harvest activities, and seed certification must be followed along the seed value chain (i.e., at the breeder seed, basic seed, and certified seed production stages) to ensure true-to-type and high quality seed. The only additional requirement for QPM seed is to perform tryptophan and protein analyses to ensure that values are above the required minimum, although in principle, QPM seed produced from pure seed stocks under strict isolation should retain the protein quality characteristics of the registered variety.

When a farmer intends to recycle seeds of a QPM OPV, special attention needs to be given to maintaining the required genetic diversity, purity, and protein quality. Another important consideration for the maintenance of an OPV is the number of plants or ears to be used. Two issues interact to determine the number:

- The number of plants or ears required to adequately represent an OPV.
- The amount of seed required to meet future needs, without having to reproduce it very frequently.

The number of plants or ears that can be taken as representative of an OPV depends on the genetic variability present within the OPV. Theoretical considerations as well as the experience of national and international programs indicate that 200-300 plants and ears would be sufficient to represent an OPV. During the maintenance process, apart from maintaining its genetic variability, it is important to ensure the protein quality through lab analysis, at least after every three planting seasons.

4.1 Preventing QPM grain contamination in farmers' fields

The *opaque2* gene must be homozygous recessive (*o2o2*) in a QPM genetic background for deriving high lysine and tryptophan content. Inadvertent pollination of a QPM cultivar by non-QPM (dominant *O2* gene) pollen makes the harvested grain non-QPM, i.e., grains on a QPM ear that are fertilized by pollen from a CM plant will not be QPM. It is very likely that a farmer's field planted with a QPM cultivar for grain production will be surrounded by plots of non-QPM cultivars (Figure 12). Therefore, QPM grain production (both hybrids and OPVs) in farmers' fields runs the risk of pollen contamination, depending upon the QPM plot size, environmental conditions (e.g., wind direction), number of surrounding plots or farms planted with non-QPM varieties, and the relative flowering dates of the adjacent QPM and non-QPM plots/farms.

The effects and significance of the contamination of QPM grain through outcrossing with adjacent non-QPM plots were studied in Ghana and Zimbabwe in plots considered representative of typical on-farm plot size. In each country, a field (0.4 ha or 0.21 ha) of a white-grained QPM variety was completely surrounded by a yellow-grained, non-QPM cultivar of the same maturity. Contamination was observed and estimated by the number and percentage of yellow kernels (evidence of pollination by yellow maize) on QPM ears at various distances from the borders. The results showed a maximum contamination of 11% of the entire grain harvest from the plot. Contamination was highest near the QPM field borders and decreased towards the middle of the field, specifically, within 12 meters of the QPM border. There was virtually no contamination in the Ghana sites (Twumasi-Afriyie, 1996), while in Zimbabwe, high outcrossing levels (63 to 83%) were observed in the peripheral areas of the QPM plots which declined to <20% within 5 m and to <10% at 10 m from the borders (Machida et al., 2012). While outcrossing was observed on at least 60% of each of the QPM crop areas, it was not significant enough to compromise QPM grain quality based on a QPM quality index of 0.8.

In practical terms, planting a QPM field next to a non-QPM field does not significantly affect the quality and nutritional benefits of the harvested QPM grain. This was demonstrated in rat feeding experiments conducted by nutritionists in Ghana. QPM and non-QPM grains were physically mixed together in varying proportions to simulate varying levels of contamination, and then assessed both in lab analyses and rat-feeding studies. It was found that contamination caused the loss of QPM benefits only after the introduction of more than 20% of non-QPM grain into the QPM grain, a contamination level higher than what was observed in the field (maximum contamination of 11%). Machida et al. (2012) suggested that farmers will not lose the benefits of QPM under normal farming conditions if there are non-QPM plots in the vicinity. Nevertheless, there are precautions farmers can take to minimize contamination, including the following:

- Since most contamination occurs on the perimeter of the plot, planting QPM in relatively square plots will minimize the length of the perimeter facing the CM plots and therefore minimize contamination.
- Plant QPM plots upwind from CM plots.Harvest the relatively pure QPM grains from the middle of the field where the proportion of QPM to non-QPM grains is higher; treat the 5 m of border rows or plants growing adjacent to CM plots as non-QPM.
- Where the length of the cropping season permits, plant QPM varieties having different maturity, so that the flowering periods do not overlap with CM varieties planted in adjacent fields.

As awareness of QPM spreads and as more farmers and entire communities start growing QPM cultivars, the problem of contamination will be significantly minimized. When QPM was first commercialized in Ghana, entire villages were saturated with QPM seed such that virtually all maize producers in the community grew only the QPM variety, thus avoiding the possibility of contamination (Twumasi-Afriyie et al., 1996).

4.2 Recycling of QPM seeds

It is important to differentiate the issue of QPM grain contamination from QPM seed contamination. Since hybrid grain is not used as seed for planting the next season, the only major concern in hybrid grain production fields is retaining protein quality in the grain for human consumption. But in OPVs, the concern is retaining protein quality both for human consumption and for use as seed for next planting. As discussed above, an advantage of using OPV seed is that farmers can save part of their seed for the next planting for about three cycles. However, when contaminated seed is sown, the non-QPM off-types in the batch will outcross with QPM plants within the plot, generating more non-QPM plants and increased contamination. Repeated recycling will quickly multiply the effect, and the seed and grain produced will very soon fail to qualify as QPM and be of no nutritional benefit to the consumer. What should a farmer do to save seed from his/her QPM grain production for the following cycle? The following measures should be taken when saving QPM OPV seeds for the next planting season:

- Farmers should select OPV seeds from the middle of their fields (see Figure 12), at least 20 m away from the QPM field borders with other maize fields, including fields planted with different QPM varieties. Consequently, the QPM seed should be harvested from the middle of a relatively large field (with 20 m border areas, a minimum size of 50 m × 50 m, or 0.25 ha, is recommended);
- Farmers should save at least 300 ears and the shelled seed from these ears should be thoroughly mixed.
- Farmers should purchase fresh QPM OPV seeds from seed producers after using their own seeds for not more than three planting cycles.

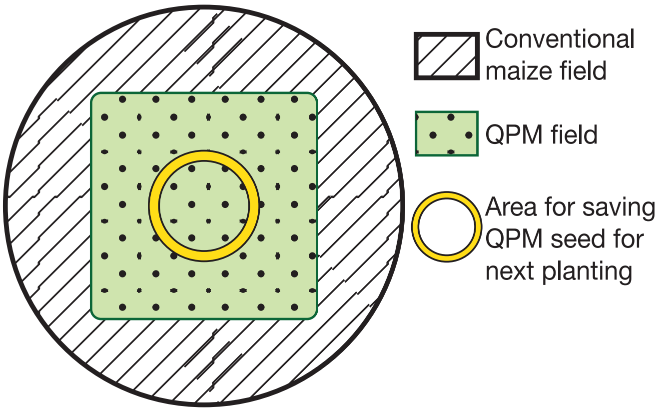


Figure 12. Schematic representation of a QPM OPV field surrounded by conventional maize fields under small-scale farm conditions. QPM OPV seeds to be used for the next planting should be taken from the middle of the QPM field in order to reduce the possibility of contamination with pollen from conventional maize varieties in the surrounding fields.

- Grains on a QPM ear fertilized by pollen from non-QPM maize are non-QPM.
- Contamination from a non-QPM field planted next to a QPM field is relatively low, ranging from 0 to 11%, and does not render the entire harvest non-QPM.
- Contamination causes loss of QPM benefits only after the introduction of more than 20% of non-QPM grain into the QPM grain.
- To protect seeds from all possible sources of contamination, farmers should select their OPV seeds from the middle (>20 meters away from all sides) of their fields.
- Farmers should purchase fresh seed from seed producers after a maximum of three planting cycles of using their own seeds in order to maintain varietal purity in the case of OPVs. In case of hybrids, they should purchase fresh seed every year to minimize contamination as well as loss of yield potential due to out-crossing.

5. QPM field management/agronomy

Agronomic management in QPM production is similar to that of CM production. Hence, only brief guidelines on the subject are given in this guidebook. Readers are advised to refer to other CM production manuals for any missing information.

5.1 Spacing and plant population density

Maize should be planted in rows in a manner that maintains appropriate plant density and spacing. The choice of plant density and spacing depends on several factors such as date of maturity, plant geometry, agro-ecology in which the variety is to be grown, and moisture availability. Early-maturing varieties are planted with narrower spacing than medium- or late-maturing varieties in mid-altitude agro-ecologies; varieties with erect leaves are more densely planted than non-erect varieties; and under moisture stressed conditions, plants are more widely spaced than in areas with adequate moisture. Accordingly, research-recommended plant densities for varieties adapted to the major maize growing agro-ecologies of Ethiopia are summarized in Table 3. Since 2014, however, sowing two maize seeds per hill at 80 x 45 cm for late- and medium-maturing varieties and 80 x 40 cm for early-maturing varieties is recommended in the maize extension package guide.

To achieve the optimum plant population on farmers' fields, the following conditions are necessary:

- The seed must be of high quality with high germination percentage.
- Optimum sowing depth must be used to ensure that the germinated seedlings penetrate the soil surface before they exhaust their nutrient/energy reserves.
- There is adequate soil moisture and good soil-seed contact to ensure uniform germination.

Providing high quality seed is the fundamental responsibility of seed companies. If there is concern or uncertainty about the quality of the seed obtained, germination tests should be conducted in advance of planting. Additionally or alternatively, as the case may be, an additional seed should be planted per hill and the extra plant is thinned two weeks after emergence if all the seeds in the hill emerge.

The farmer should also plant the seeds at a uniform soil depth to attain uniform germination and seedling emergence for optimum plant population. Sowing depth depends upon soil type, soil moisture content, and seed size. If the seed is planted too deep, the seedling depletes its food reserves before it emerges through the soil surface. On the other hand, shallow sowing exposes the seed to damage by animals, birds, and insects as well as desiccation if a dry spell follows planting and there is insufficient moisture to trigger seed germination. The optimal sowing depth varies with seed size. Generally, a depth of 5-7 cm is recommended. However, for smaller seeds (such as those of BHQPY545), a depth of 3-5 cm is recommended. The seed should be covered with soil and the soil tamped down in such a way that good contact between the seed and soil is obtained. This will ensure that the seed is able to imbibe moisture easily and that all seeds in the plot imbibe and germinate at the same time.

Table 3. Recommended plant density and spacing of different QPM varieties in different agro-ecologies of Ethiopia.

Variety	Spacing (cm)	Plant density	Suitable agro-ecology
AMH760Q	75 x 30	44,444	Mid-altitude sub-humid agro-ecology
BHQP542	75 x 30	44,444	Mid-altitude sub-humid agro-ecology
BHQPY545	75 x 30	44,444	Mid-altitude sub-humid agro-ecology
MHQ138	75 x 25	53,333	Low moisture stress areas
Melkassa 1Q	75 x 20	66,666	Low moisture stress areas
Melkassa 6Q	75 x 25	53,333	Low moisture stress areas

5.2 Soil nutrition

The maize plant requires different nutrients in different amounts, depending on soil type and environmental conditions, for optimum growth and yields. Suboptimal levels of essential nutrients will result in less than optimal yields. Deficiencies of the major nutrients, nitrogen (N), phosphorus (P) and potassium (K), can be recognized by characteristic symptoms on the leaves, as shown in Figure 13. Nitrogen deficiency gives rise to young plants that are pale, light green, or yellow in color. As the deficiency progresses, premature yellowing starts at the tips of the lower leaves and moves along the mid-vein of the leaf until the entire leaf appears necrotic (brown and dry or dead). Maize plants suffering from N deficiency produce small ears with unfilled kernels (especially at the tip of the ear) and reduced protein content. Phosphorus deficiency symptoms include stunted growth, dark green or reddish-purple leaves, particularly at the leaf tips of young plants, and delayed flowering and ripening. In P-deficient maize, ears are small, often twisted, and have undeveloped kernels. In contrast to N deficiency, K deficiency symptoms appear on the leaf margins as yellowing and firing, progressing from the tip of the leaf to the base as the severity increases.

Nutrient deficiencies are corrected by the application of appropriate fertilizers. Current fertilizer recommendations in Ethiopia include only N and P. For the past few years, however, there has been an ongoing effort to develop blended fertilizers based on macro- and micro-nutrients. The soil fertility status atlas developed by the Ethiopian Soil Information System (EthioSIS) indicates that Ethiopian soils are also deficient in sulphur (S), boron (B), zinc (Zn), potassium (K), and copper (Cu) in addition to N and P. Accordingly, new fertilizer blends targeting the missing nutrients have been formulated and are being verified in farmers' fields before wide-scale dissemination. Upon completion of these verification trials, site-specific multi-nutrient based fertilizer application recommendations are expected to be released. Please refer to Section 7.6 for N and P fertilizer application rates.

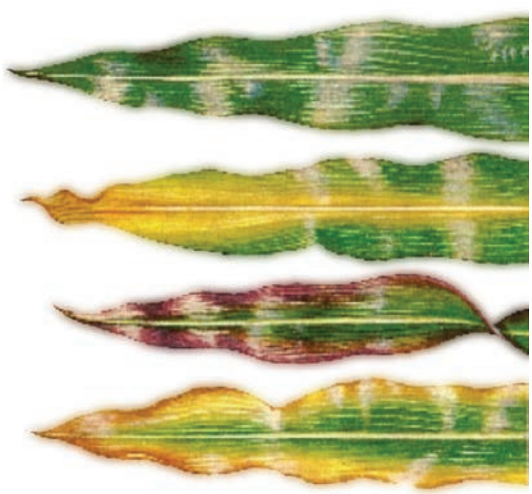
5.3 Pest management

5.3.1 Weeds and weed management

Successful cultivation of maize, whether conventional or QPM, depends largely on effective weed control. Competition by weeds for nutrients, moisture, and radiation can result in very large losses in maize grain yield. Timely weed control during the first six to eight weeks after planting is crucial. Weeds can be controlled using various methods including hand weeding, cultivation and application of appropriate herbicides.Hand weeding, the most commonly used method among Ethiopian farmers, is recommended at two critical stages:

- The first weeding should be carried out 14 to 21 days after sowing or at the three-leaf stage. At this stage the nodal root of maize has not developed enough to compete against weeds.
- The second weeding must be done four to six weeks after planting or at the five-leaf stage before urea is applied.

Pre-emergence herbicide such as Primagram Gold® at a rate of 4 L/ha can be used to prevent the emergence of weed seeds early in the season. Post-emergence herbicides such as 2-4-D can be sprayed at the rate of 2 L/ha to control broadleaf weeds. Generally, farmers should use an integrated approach to weed



a healthy corn plant leaf is deep green and glossy

a leaf from a plant with nitrogen deficiency yellows down the midvein starting at the tip and moving back towards the stem

a leaf displaying phosphorus deficiency turns red-purple along the leaf margins

a leaf from a potassium-deprived plant features firing and yellowing along the leaf margins

Figure 13. Maize leaves showing nitrogen, phosphorus, and potassium deficiency.

management that combines all available options (herbicide, crop rotation, use of recommended spacing, timely planting, hoeing and hand pulling, improving soil fertility, and use of weed-free seeds). The aim is to keep weed numbers low and prevent them from producing seeds throughout the cropping cycle.

5.3.2 Major maize diseases and their management

Diseases reduce maize yield and grain quality; the degree of loss depends on the severity of the infestation and the growth stage at which it occurs. Several maize diseases, both fungal and viral, infect maize in Ethiopia. Only the major ones are discussed in this manual. The best approach to control maize diseases is through developing and deploying resistant varieties. Resistance breeding is a continuous effort in which new high-yielding varieties with increased resistance are developed to replace older varieties whose resistance is broken down by the rapid evolution of the pathogens. In the absence of resistant varieties, and to reduce disease pressure on existing resistant varieties, various cultural practices common to some or all of the above diseases should be used, including:

- Rotation of maize with legumes or other non-cereal crops as planting maize after maize in the same field enhances pathogen build-up.
- Using deep tillage to bury infected plant residues that carry disease inoculum.
- Controlling all the alternate hosts of the pathogen.
- Removal and burying or burning of infected maize plants (in case of MSV and MLN) at an early stage of crop development.

a. **Turcicum leaf blight (TLB*):*** TLB is a fungal disease. Leaves are first affected by small, diamond-shaped lesions that elongate as they mature. The final lesion is rectangular and 2-3 cm long. Lesions are light-brown in color with a reddish-brown border and a light yellow ring around them. Lesions may merge, completely burning large areas of the leaf. This may lead to stalk and cob rot, which can cause significant yield loss. The pathogen survives on/in infected leaves, husks, and other plant parts.

b. **Gray leaf spot (GLS):** GLS occurs in the warm to hot areas of Ethiopia, especially during the humid season. Lesions are pale brown or gray to tan in color, long, narrow, and rectangular, characteristically restricted by the veins. Losses have been severe in some maize growing areas in recent years. The fungus survives in maize residues, and spores develop to initiate new infestations when weather conditions are conducive. Spores can be carried over long distances by the wind. Secondary spread of the disease within and between fields occurs by conidia produced from lesions.

c. **Maize streak virus (MSV):** MSV is transmitted by leafhoppers which are harbored by several wild and cultivated grass families. The initial symptoms of the disease are small, whitish spots, which become colorless streaks running parallel to the veins along the entire length of the leaf. When the plant is infected at the seedling stage, this streaking appears on all except the lowest leaves. Infected plants become stunted and produce smaller than normal ears.

d. **Common leaf rust:** Rust appears as small, round to oval, brown or rusty orange pustules initially on the lower leaves, before spreading to the upper parts. Brown to black circles may appear around the pustules. Severely

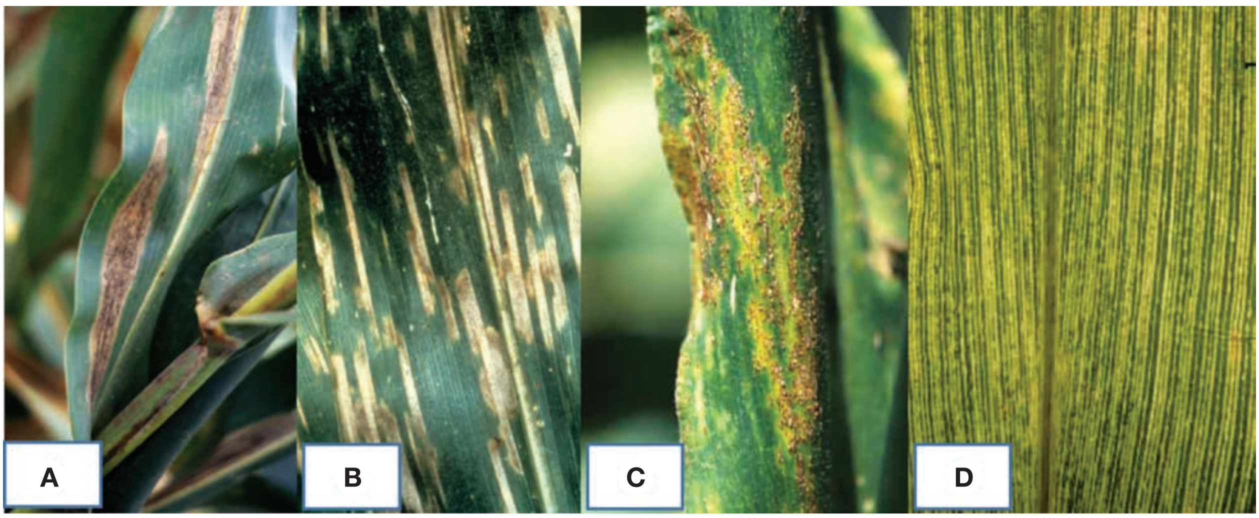


Figure 14. Symptoms of common foliar diseases in maize. A) TLB; B) GLS; C) common leaf rust; D) MSV.

affected leaves turn yellow and die early. Ears of severely affected plants are much lighter than normal and the seeds are pinched and loosely attached to the cob.

e. **Stalk and ear rots**: Different species of fungi (*Fusarium* and *Gibberella* spp.) produce stalk and ear rots. Whitish-pink cottony fungal growth develops on and between the kernels and sometimes on the silks. Infected plants are weakened and break easily during strong winds and rains. Mycotoxins, which are harmful to humans and livestock, are also produced on the ears. These diseases can be controlled with the use of optimum plant populations and application of adequate nitrogen fertilizer.

f. **Maize lethal necrosis (MLN):** Since 2011, MLN has emerged as a major threat to food security in eastern Africa. The disease is a result of infection of a maize plant by the *Maize Chlorotic Mottle Virus* (MCMV) and any of the cereal viruses in the Potyviridae group, especially *Sugarcane Mosaic Virus* (SCMV). Between 2011 and 2014, MLN was reported by most countries in east Africa, including Kenya, Tanzania, Uganda, Rwanda, D.R. Congo, and Ethiopia. In Ethiopia, MCMV and MLN were reported for the first time in 2014 (Mahuku et al., 2015).

Maize plants infected by MLN show chlorotic mottling on the leaves, usually starting from the base of the young leaves in the whorl and extending upward towards the leaf tips. Advanced stages of the disease are reflected by necrosis of the leaf margins and progressing to the midrib, stunting of the plant, and eventual necrosis (drying up) of the leaves and the whole plant. Plants that are affected at later growth stages show chlorotic mottling on the leaves and dry leaves starting from the top, and either show barrenness (with no ear formation) or poor seed set. Fungal infections are also often observed on MLN-affected plants, and severely affected plants often produce diseased ears and low quality grains that are unfit for consumption (Prasanna, 2015). MLN-causing viruses are transmitted individually in the field from infected maize plants or other co-hosts of MCMV and SCMV by insect vectors. MCMV was also shown to survive in maize crop residues. MCMV and SCMV can also be either seedborne (= seed produced by an infected plant can carry the virus) or seed-transmitted (= virus can pass from infected seed to a newly generated plant). It is becoming clear that seed transmission of MCMV is playing a role in the rapid emergence of MLN across eastern Africa.

More than 95% of the commercial maize varieties in eastern Africa were found to be vulnerable to MLN. Five MLN-tolerant maize hybrids developed by CIMMYT were approved for commercial release in Kenya, Uganda, and Tanzania in 2013/2014, and are in the process of seed scale-up for commercialization. Intensive efforts are also underway at CIMMYT to develop new breeding materials as well as elite hybrids that combine drought tolerance, nitrogen use efficiency, and MLN tolerance/resistance. In addition to host-controlled resistance, the best approach for MLN management is integrated pest management practices encompassing cultural control (such as crop rotation, crop diversification, and good field sanitation) and vector control using seed treatment followed by foliar sprays. Seed dressing of 100 kg (one quintal) of seed with 120 ml of Gaucho® diluted in 1.5 liters of water (to enhance chemical coating of the seed surface) may control vectors that transmit the virus from plant to plant.

Figure 15. Various symptoms of maize lethal necrosis (MLN).

5.3.3 Major insect pests of maize and their management

Insect pests are among the most important biological factors that limit maize production. The first step in managing insect pests is to identify the insect, determine its population in the field, and establish the extent of infestation and the damage it is causing. Some of the common and economically important insect pests of maize in Ethiopia are described below.

a) Maize stem/stalk borers*:* Stem borers are the most common and widespread pests of maize in Ethiopia. Common species are the maize stem borer (*Busseola fusca*) and the spotted stem borer (*Chilo partellus*). Damage is caused by the feeding of young larvae, which cause more serious damage at the seedling stage of maize. The initial symptom of infestation on young plants is rows of oval perforations on leaves of the unfolding whorl. As they develop, the larvae tunnel into the leaf midrib, damage the growing point causing a condition referred to as "dead heart," and bore into the stem. Management of stem borers includes:

- Early sowing.
- Cutting and laying maize stalks thinly and horizontally in the field for weeks (before stacking them for future use) to kill the pupae and reduce the subsequent generation of borers.
- Destruction of crop residues to kill pupae left in old stems and stubble and prevent carry-over populations.
- Intercropping maize with crops that are non-hosts for stem borers (e.g., cassava and grain legumes).
- Intercropping maize with a repellent plant such as desmodium, and planting an attractive trap plant, such as Napier grass, as a border crop to draw stem borers away from the maize crop (known as "push-pull" strategy).
- When infestation is severe, application of cypermethrin (1%) or diazinon (10%) granules at the rate of 3-5 kg/ha to the leaf whorl in the early stages of crop growth to kill early larval instars. This method has limited effectiveness once the larvae bore into the stem.

b) Termites: Different species of termites attack maize at different growth stages and are difficult to control because they attach themselves to the crop beneath the soil surface. The following methods are recommended to minimize the damage:

- Destruction of termite mounds/nests by physical means (including deep plowing) or poisoning with Malathion® at the rate recommended by the manufacturer.
- Dressing seeds with insecticides: fipronil (Regent 500 FS®) at a rate of 8-13 ml/kg, and SeedPlus 30 WS® at 5 g/kg seed.
- Spraying Diazinon 60% EC® at 2.5 L/ha and GUFOS® (chlorpyrifos 48%, also called Dursban®) at 200 ml/ha.Crop rotation with less susceptible crops (e.g., sorghum is known to be less susceptible than maize).
- Prompt harvesting.

c) African armyworm (*Spodoptera exempta*): The African armyworm is a sporadic but very damaging pest, capable of destroying an entire field in a matter of weeks. They are small, dark green caterpillars hatched from eggs laid, usually on the underside of leaves, by a dark gray nocturnal moth. Young crops should be checked daily if there is any sign of an outbreak elsewhere in the country. For effective control, caterpillars must be sprayed when they are still small; once mature (about 3 to 3.5 cm long), they may have already caused serious damage and it may no longer be economical to treat the crop. Different organophosphate insecticides (e.g., Malathion 50% EC® and fenitrothion 50% EC®, each at 2 L/ha, fenitrothion 95% ULV® at 1.5 L/ha, or diazinon 60% EC® at 1 L/ha), and carbamate insecticides (carbaryl 85% WP® at 1.5 kg/ha) are recommended.

d) Cutworms (*Agrotis* spp.): Cutworms damage maize plant at the seedling stage. The larvae cut maize seedlings at or a little below ground level, mostly at night. They remain sheltered below the ground during the day. Removal of the soil around cut or injured seedlings reveals greasy or oily worms which are grayish, brownish, or black in color. When disturbed, the larvae curve their bodies into a "C" shape and remain motionless for a short period. Methods recommended to minimize cutworm damage include:

- Early weeding, at least two weeks after planting;
- Plowing and harrowing the field to expose cutworms to natural enemies and desiccate them; and
- Destroying plant residues that could harbor cutworms.

The effects of cutworms can be reduced by dressing seeds with chemicals (any recommended seed dressing) or through the application of soil insecticides such as Imidacloprid® at 56-140 g/ha.

6. Post-harvest management

6.1 Grain storage

To maximize the benefits of improved production from improved maize varieties and crop management, attention must be paid to proper post-harvest storage. Yield losses of 30% or more can occur when grain is not protected from storage pests or fungal diseases. The ideal moisture content for storing maize is 12.5-13.0%. Inadequate drying can lead to the development of fungal diseases, as described below.

After drying, seeds should be stored in a clean storage container in a cool place. Maize can be stored as cobs (with or without husks) on elevated racks or in cribs, or in the form of shelled grain in containers. Storage containers most commonly used by farmers in Ethiopia include the *gotera*, the *gotha*, sacks, bags, and pits dug into the ground. Since these traditional storage methods have limited utility and often experience substantial losses, the use of modern containers is strongly advised. Effective storage against insect pests also depends on the use of residual insecticides such as Actellic 2% dust or Malathion 5% dust, or a fumigant such as aluminum phosphide tablets, or on the exclusion of oxygen to suffocate the pests. Small, closed storage bins constructed from wood, bricks, or concrete, or metal silos may be effective if they are airtight. Metal silos and Super-grain® bags made of heavy-duty impermeable plastic were demonstrated in some parts of Ethiopia by SG 2000 with funds from CIMMYT.

Like CM, some QPM varieties may be susceptible to storage pests. The most common post-harvest pests are briefly discussed below.

a) **Insect pests:** Insects are generally the most serious pests of stored grain. The most frequently encountered and economically important insect pests of stored maize include:
   - Maize weevil (*Sitophilus zeamais*) and rice weevil (*S. oryzae*)
   - Angoumois grain moth (*Sitotroga cerealella*)
   - Larger grain borer (*Prostephanus truncatus*)
   - Flour beetles (*Tribolium castaneum* and *T. confusum*)
   - Rusty red grain beetles (*Cryptolestes* spp.)
   - Indian meal moth (*Plodia interpunctella*)
   - Warehouse moth (*Ephestia* spp.)

Maize and rice weevils, the larger grain borer and the Angoumois grain moth (Figures 16, 17, and 18) start infesting maize before harvest and are referred to as primary pests. Important cultural practices that help to control storage pests include:

- selecting grain that is not infested
- proper drying before storage
- maintaining storage hygiene
- cleaning of storage containers before depositing the grain
- locating *goteras* far from maize fields to avoid cross infestation from the field to store or viceversa

Figure 16. Maize weevil.

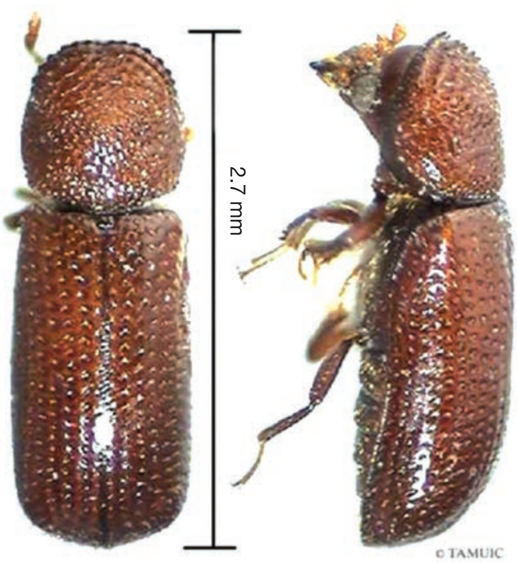


Figure 17. Larger grain borer (dorsal and side views).

Figure 18. Angoumois grain moth.

- mixing the grain with residual insecticides such as Actellic (Pirimiphos-methyl) 2% dust or Malathion 5% dust at the rate of 25-50 g per quintal
- application of fumigants such as aluminum phosphide tablets to provide rapid control of existing insect populations

b) Storage diseases: Fungi, the most widespread disease-causing agents affecting stored grain, appear as mold on the affected ear or grain. Infected grains may lose color, viability, and food value and may contain mycotoxins which can poison both people and livestock.

Sorting out diseased ears during harvesting and storage, drying the grain to the recommended moisture level before storage, and keeping storage containers cool and dry will reduce the incidence of post-harvest pathogens significantly.

c) Rodents*:* Rodents, mainly rats and mice, cause heavy losses of stored grains. In addition to the direct damage they cause by eating, they contaminate the grain with their urine and feces. Because it is very difficult and costly to separate these contaminants, most of the stored maize grain will be unfit for human consumption. The most effective means of controlling damage to stored grain by rodents is the use of sealed containers such as metal silos. In their absence, fitting elevated containers such as crib-style grain stores with rat baffles is recommended. However, these improved structures may be prohibitively expensive for adoption by many farmers. The use of rat traps or keeping cats can reduce the population of rats except acute or chronic infestations. Keeping the storage area free from trash and spilled grains that may attract or harbor rats is also advisable. Rodenticides such as zinc phosphide (4%) mixed with potatoes or carrots as per the manufacturer's recommendation or fumigation (aluminum phosphide at a rate of 5 to 10 tablets per ton of grain) can also be employed.

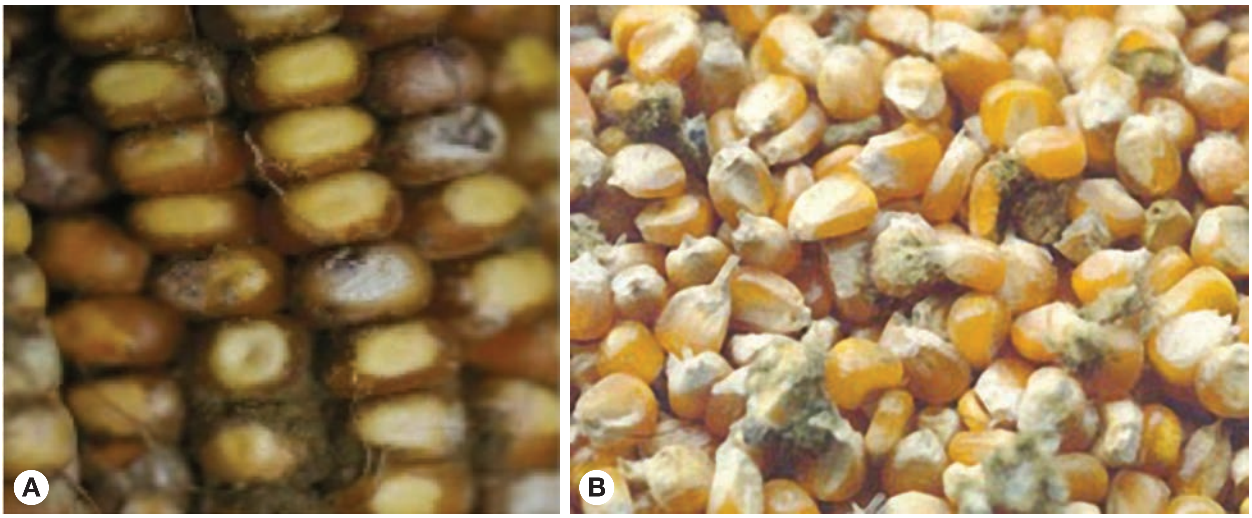


Figure 19. Maize ear (A) and grains (B) infected by the fungus *Aspergillus flavus*.

7. Conducting QPM field demonstrations

Since QPM varieties are relatively new to Ethiopian farmers, an important prerequisite to their adoption is raising awareness among male and female farmers with regard to QPM varieties, their characteristics and, most importantly, the nutritional benefits that can be obtained from their consumption, especially by young children and lactating mothers. The most common way of introducing new varieties and agricultural technologies to farmers is by establishing widespread, on-farm demonstration plots. Demonstrations must be carefully conducted to present the varieties under the best management practices so that the maximum yield potential of the varieties is attained. Farmers will not adopt a variety if the grain yield of a demonstrated new variety is not the same or better than that of the varieties they already grow. In order to achieve good results, careful attention must be given to the selection of demonstration plots and participating farmers, proper layout of demo plots, selection of appropriate check varieties to which the introduced variety will be compared, and best management practices including timely and proper planting and thinning, weeding, fertilizer application, and pest management. Frequent monitoring and evaluation of the demonstration plots ensures favorable introduction of QPM varieties to farmers and encourages farmers to try and adopt them. The major steps for conducting field demonstrations and field days are described below.

7.1 Selecting demonstration plots

Demonstration sites are selected by trained woreda staff and/or extension agents who understand the significance of the demonstrations very well. The site should also be suitable for growing the QPM variety to be demonstrated. In addition, the following conditions should be satisfied:

- Site uniformity – the site should be free of tree stumps, termite mounds, obvious fertility gradients, depressions, etc., that could affect the relative performance of the demonstrated variety and the check. Review the previous history of the site with the plot owner to identify any indication of non-uniformity by asking the following questions: Was the entire site under the same crop in the previous season(s)? Did the farmer notice any irregularities in the field? Was the drainage good, or was there waterlogging on the site? Also, look for any irregularities in soil texture and color across the site.
- Weediness – the site should not be excessively weedy.
- Soil properties – the soil should have good tilth, structure, organic matter content, uniform texture and color, good drainage/ permeability, etc.
- Topography – the site should not be very sloping (a slope <12%); the slope should be unidirectional for effective blocking of the demo plots.
- Size/area – the area should be adequate to accommodate plots on the same contour. Using the recommended plot size of 50 m × 25 m (see section 7.3 below) with four varieties (two QPM plus two checks) requires an area of 2000 m^2. The dimensions will depend on whether the site is sloping or not (see below).
- Location – the plots should be visible from a nearby road or located along a well-traveled pathway. They should also be easily accessible to visitors and field day participants without having to cross other fields belonging to the farmer or his/her neighbors.

7.2 Selecting farmer cooperators

Farmers who will host demonstrations should be selected based on the availability of suitable land (see above), and their willingness to implement improved agronomic practices properly and on time as per training and advice from extension agents. They must also be willing to allow other farmers to visit their plots during field days and on other occasions. Women should be encouraged to host demonstrations so that they can learn about the technology and its benefits directly from extension agents and then transfer their knowledge to other women. If women know and understand the nutritional benefits of the technology, they will be better able to persuade their husbands to adopt QPM and share in those benefits in an equitable way.

Different strategies should be applied to increase the participation of women, including:

- supporting women-managed demonstration plots in male-headed households;
- identifying woman-headed households and explaining the agronomic and nutritional benefits of QPM technologies to convince them to host the demonstrations;
- delivering seeds and other inputs on time as women have less access to inputs; and
- developing convenient time frames for women, considering the constraints they face because of their multiple roles.

7.3 Demonstration plot layout

The layout or arrangement of plots at the demonstration site depends on the gradient of the site and the number of QPM varieties and CM checks to be included in the field demonstration. Alternative plot layouts recommended under different situations are shown in Figure 20. On slopy sites, rectangular plots should always be used, with the longer plot dimension placed perpendicular to the slope (i.e., along the contour) and the shorter dimension falling along the slope. Seeds should be planted in rows along the contour lines. When demonstrating two or more QPM varieties in a slopy field, the following options can be used based on the particular features of the field and the number of varieties to be tested.

- Two QPM varieties of the same maturity or yield potential (i.e., comparisons between QPM varieties and with checks) – plant all varieties on the same contour (Option B; see Figure 20) with each QPM variety beside its check and beside the other QPM variety. This will permit a side-by-side comparison of one QPM variety with the other, as well as with the check.
- Two QPM varieties having different maturities or yield potentials (i.e., comparisons between QPM varieties not important) – use Option B; but if the dimension of the land along the contour is not sufficient for laying four plots horizontally, planting each QPM variety and its respective check on different contours (as in Option A, Figure 20) is appropriate.
- Two QPM varieties and their checks on flat land – either Option A or B can be used.
- When demonstrating two QPM varieties of similar maturities and yield potentials with the same check – Option C (see Figure 20) can be used instead of planting the same check twice.

Plot size for field demonstrations depends on the availability of land, seed, and labor. Most farmers do not like their fields to be divided into small plots. On the other hand, larger plots may be difficult to manage properly. A larger plot size allows making a good visual comparison of the QPM variety with the check and/or with another QPM variety. It also gives farmers the opportunity to see and harvest QPM grain from the middle of the plot(s), which is less contaminated by pollen from surrounding CM fields. A good compromise, considering all these factors, seems to be a plot size of at least 5000 m^2 (25 m x 20 m) for each variety. In exceptional circumstances, where area or seed is limiting, smaller plot sizes may be used (although not recommended); the minimum size should be 10 m × 10 m.

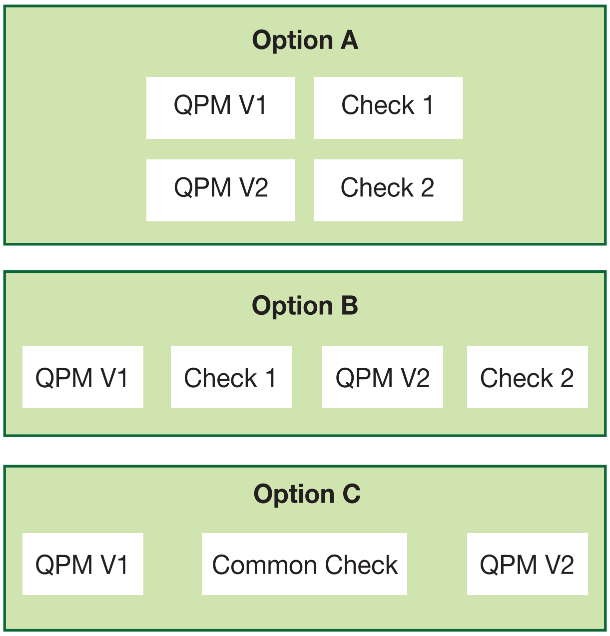


Figure 20. Options for the arrangement of QPM varieties and conventional check plots.

7.4 Choice of QPM and control varieties

As discussed in Section 3, the QPM varieties released in Ethiopia have specific areas of adaptation where they express their maximum genetic potential and tolerate relevant biotic and abiotic stresses. Therefore, when planning field demonstrations, it is important to select QPM varieties that are suitable for the target area. Refer to Section 3 to choose a QPM variety adapted to the major maize agro-ecologies.

In most cases, the check variety will be an improved conventional variety popular in the area. If there is no widely-grown improved variety, on the odd occasion, a farmers' variety can be used as a check. The criteria for choosing a check variety are:

- It should be widely produced or known by farmers.
- It should have the same maturity as the QPM variety to be demonstrated.OPV checks should be compared with QPM OPVs and hybrid checks with QPM hybrids.

7.5 Planting and thinning

A seed rate of 25-30 kg per hectare, depending on seed size, is required for maize to attain the recommended density, assuming good germination and no losses. Demonstration plots should always be planted to achieve the recommended plant density for maximum yields. To compensate for possible losses due to poor germination or emergence, insect pests, ground squirrels, erratic rainfall at planting, etc., plots should be planted with either one extra seed per hole or one extra seed per alternate hole; plots must be thinned to the recommended density no later than three weeks after planting, once the seedlings are well established (Table 5).

Please refer to Section 5.1 for the recommended spacing and sowing depth. When thinning, always consider two adjacent holes together and thin out the number of extra plants per two holes, as indicated in Table 5. However, in view of the recent recommendation of planting two seeds per hill, one has to plant more than two seeds per hill to guarantee the emergence of more than two seedlings per hill and thin them down to two per hill later.

7.6 Fertilization

The fertilizer recommendation currently in use is only for nitrogen (N) and phosphorus (P), sourced from urea (46% N) and DAP (di-ammonium phosphate; 18% N and 46% P_2O_5). Fertilizer rates for QPM production do not differ from those used to produce CM. A multi-nutrient fertilizer blend will soon be publicized for wide-scale dissemination, once it is verified by EIAR and officially adopted by the MoA and BoA.

Rates of fertilizer application: Current location-specific N and P fertilizer recommendations for use in QPM demonstrations, according to variety and target area or agro-ecology of the major maize growing zones, are presented

Table 4. QPM varieties and suggested conventional checks for field demonstrations.

No.	QPM Variety	Conventional check variety
Hybrids		
1	AMH760Q	BH660
2	BHQPY545	BH540
3	MH138Q	(i) BH540 (in high potential transitional or mid-altitude agro-ecologies) (ii) Melkassa 2 or a farmers' cultivar (in drought prone agro-ecologies)
OPVs		
4	Melkassa 1Q	Melkassa 1 or a farmers' cultivar
5	Melkassa 6Q	Melkassa 2 or Melkassa 4 or farmers' cultivar

Table 5. Seeds planted per hill, emergence, and thinning intensity to maintain the required plant density.

Seeds planted per two holes	Seedlings emerged per two holes	Thinning action
4	4	Remove 1 per hole
	3	Remove extra seedling from a hole having 2 plants
	2	Leave both seedlings regardless of holes
3 (1 extra seed per alternate hole)	3	Remove extra seedling from a hole having 2 plants
	2	Leave both seedlings regardless of holes

below (Table 6). The same application rate must be used for both the QPM variety and the check variety, which must be managed in the same way.

Timing and method of fertilizer application: In all cases, the complete dose of DAP is applied at planting. Direct contact between fertilizers and seeds must be avoided; fertilizer should be placed below or to the side of the seed. Open the planting hole (10 cm deep), place the fertilizer in it and cover with 5 cm of soil; place the seed on top or on the side of the planting hole and cover with soil to the desired depth. Urea fertilizer can be applied all at once or split into two applications, depending on the agro-ecology:

- In highland areas, apply urea in three splits: one-third at planting (with the DAP), one-third at knee height (8-10 leaf stage), and one-third at flowering.
- In mid-altitude, sub-humid agro-ecologies, apply the urea in two splits: one-half at planting and one-half at knee height.
- In moisture stressed areas, the full dose of urea should be applied at knee height.
- In all cases, spot apply the side-dressed fertilizer 3-5 cm away from the seed/plants to avoid seed burn and improve fertilizer use efficiency.

7.7 Pest management

Pest management in demonstration plots should be undertaken as required according to the recommendations in Section 5. In order to present the varieties and the technology in the best possible light, all pest management operations must be conducted in a timely and efficient manner.

Weeds and weed management: If hand weeding, perform the first weeding 14 to 21 days after sowing or at the three-leaf stage; the second weeding is carried out four to six weeks after planting or at the five-leaf stage, before the application of urea. For chemical weed control, apply pre-emergence herbicide (Primagram Gold® at a rate of 4 L/ha) to prevent the emergence of weed seeds early in the season. Apply post-emergence herbicides such as 2-4-D at a rate of 2 L/ha to control broadleaf weeds. Supplement chemical control with hand weeding, as required, to ensure clean plots and optimal crop growth and yield.

Disease management: Disease control depends, for the most part, on the host-plant resistance of the demonstrated QPM varieties and their checks. In the case of ear rot on BHQPY545, where the crop matures during extended rainfall and moist conditions, consideration should be given to turning down the ears on the demo plots to prevent the ingress of water and reduce the possibility of infection.

Control of insect pests: Insect infestations should be controlled according to the guidelines in Section 5. Specifically,

i) **Maize stem/stalk borers**: To kill early larval instars, apply cypermethrin 1% or diazinon 10% granules at the rate of 3-5 kg/ha to the leaf whorl in the early stages of crop growth **before the larvae bore into the stem**.

ii) **Termites**: If termites are expected to be a problem at the demo site, use chemical controls to minimize the impact:
   - Dress seeds with fipronil (Regent 500 FS®) at a rate of 8-13 ml/kg, and SeedPlus 30 WS® at 5 g/kg seed; and
   - Spray Diazinon 60% EC® at 2.5 L/ha and GUFOS® (chlorpyrifos 48%, also called Dursban®) at 200 ml/ha.

Table 6. Recommended fertilizer type and rate for some locations in Ethiopia.

Type	Fertilizer rate (kg/ha)										
	Adet/ Bure	Hara-maya	Ambo	Holetta	Hawassa	Jimma	Bako	Ghimbi	Melkassa	Pawe	Gambella
DAP	150	100	100	150	100	150	150	150	100	0	100
Urea	200	150	200	200	200	200	200	150	50	150	50

Source: Wakene et al. (2011)

iii) **African armyworm (*Spodoptera exempta*)**: Check young crops daily if there is any sign of outbreak elsewhere in the region. To be effective, control must be initiated while caterpillars are still small. Spray one of the following insecticides according to the manufacturer's recommendations:

- Malathion 50% EC® and fenitrothion 50% EC® each at 2 L/ha;
- Fenitrothion 95% ULV® at 1.5 L/ha;
- Diazinon 60% EC® at 1 L/ha;
- Carbaryl 85% WP® at 1.5 kg/ha.

iv) **Cutworms (*Agrotis* spp.)**: In addition to normal cultural practices (early weeding, plowing and harrowing to expose cutworms to natural enemies and desiccate them, destroying crop residues that harbor cutworms), dress seeds with recommended seed dressing chemicals or apply soil insecticides such as Imidacloprid® at 56-140 g/ha.

7.8 Monitoring and yield data collection

Demonstration plots must be monitored frequently. Critical stages and activities that need close supervision include: site selection, land preparation, planting, seedling emergence, early and late vegetative stages, and grain-filling and maturity. Monitoring is useful to check whether recommended field practices are followed during each of the activities and, if they are not, to take timely corrective measures. Timely supervision during site selection, for example, makes it possible to change wrongly selected sites. Experience shows that close supervision significantly improves the management, and thereby, the performance of the demonstration plots. Provision of training and inputs alone does not guarantee the proper implementation of demonstrations.

A record book should be maintained for each demonstration. The site/location name/reference, host farmer's name, sketch of the plot layout, and dates of all operations (land preparation, sowing, fertilizer application, thinning, weeding, pesticide applications, etc.) should be recorded. During supervisions, observations as well as feedback from host farmers on the field performance of the QPM varieties as compared to conventional varieties should be collected and recorded, whether the QPM varieties are liked or not, together with the reasons, and other relevant information concerning adoption. Farmers in the vicinity should also be invited to the demo plots at least once to see the new QPM varieties.

Grain yield data and other relevant information on the performance of QPM varieties and the check varieties should be recorded. Grain yield should be estimated based on a minimum sample area of 50 m^2 (10 m × 5 m) from the center of each 500 m^2 (20 m × 25 m) plot following the procedure described below:

- Harvest cobs from all plants in the sampled area (50 m^2) and weigh to determine the field weight (kg); weight of all the ears from the sampled area at harvest is referred to as field weight.
- Weigh 10 randomly select ears, shell and weigh the shelled grain alone (without cob/rachis), and calculate the shelling percentage as follows:

$$\text{Shelling \%} = \frac{\text{Grain weight (kg)}}{\text{Field weight (kg)}} \times 100$$

- Determine the actual moisture content (percent) by taking the grains shelled from the 10 randomly selected ears using an appropriate grain moisture testing apparatus.
- Adjust grain moisture content to 12.5% using the following formula:

$$\text{Adjusted moisture content (\%)} = \frac{\text{100-actual moisture content (\%)}}{\text{100-12.5}} \times 100$$

- The final grain yield (t/ha) should be presented as adjusted to 12.5% moisture content using the following formula.

$$\text{Grain yield t/ha} = \frac{\text{Field weight (kg) x adjusted moisture content (\%) X shelling \% x 10}}{\text{sampled plot area } (m^2)}$$

8. Organizing field days

Two types of field days should be organized, the first at the *kebele* (smallest administrative unit) level and the second at the *woreda* or higher level. Field days are organized any time from grain-filling onwards, depending on convenience, but have to be organized at a crop growth stage when a clear comparison can be made between the QPM and CM varieties. Beside organizing field days, farmers should be encouraged to visit (farmer-to-farmer experience sharing) QPM demo plots in the *kebele* at different crop stages, starting from the green cob stage to when it gets dry. At each visit, the name and gender of farmers visiting the demos, and the issues raised by them, are noted down. These visits are means of creating confidence in the varieties' adaptation to the area and of sharing experiences on QPM field management practices with nearby farmers.

In addition to the multistage visits, field days are organized at each *kebele* to show as many farmers as possible how the QPM varieties perform compared to the conventional checks and get their feedback on the technology. Usually the host farmers are the ones who explain the work performed in raising the demos. Field day participants should be registered by name and gender to avoid counting them twice. The number of women and men farmers participating in field visits and field days (added together) should be greater than 300.

The *woreda* level field day is a larger affair than the *kebele* level field day. It may involve visits to two or three demonstrations in relatively close proximity; those visited are selected based on their management and stand appearance (very good stand establishment, vigorous growth, little evidence of pest or disease damage, etc.) so that the varieties are presented in their most attractive state. The number of visitors at *woreda* level field days may be as many as 1,000 but a minimum of 500 farmers should ideally participate. In addition to farmers, government officials (local administration, agriculture, and health bureaus), seed companies, the media, donors, and other stakeholders should be invited to attend and be available to respond to pertinent issues that farmers may raise, such as seed availability. Thus, the *woreda* level field day is also a forum where officials receive feedback from farmers and give their assurance for future inclusion of the technology in the regular extension system, input supply, etc. The organization and preparation of field days include the following important activities.

8.1 Preparing the demonstration plots

Field days at both the *kebele* and *woreda* levels should be organized around the best demonstrations, since the purpose of a demonstration is to "sell" the technology to the farmer. The demonstration plots should be selected ahead of time (at least a week before the field day) and, although plots are expected to be managed properly during the whole season, they should nevertheless be cleaned/weeded again in advance of the field day. If possible, the border and pathways around the plots should also be cleared for easy access and movement of participants. The plot to be visited should be clearly labelled with information such as the name of the variety, seed rate, planting date, and fertilizer rate used. On the day of the event, if the crop is sufficiently mature, it is good to remove husks of three or four ears (while hanging on the maize plant) on the border rows to depict the color, size, and other aspects of the grain /kernel.

8.2 Promoting the field day

8.2.1 Announcements and invitations

Field day plans should be communicated to partners and stakeholders well ahead of time due to the busy schedules of officials at different levels. The dates of field days should be selected in consultation with the *woreda* BoA staff and other stakeholders, as necessary. The time should be suitable for all participants, especially to the farmers, with due consideration to the convenience of women farmers in both male and female-headed households. Organizing field days on market days is not suitable for farmers and should be avoided as much as possible. Development agents (DAs) in the *kebele* are responsible for informing and reminding farmers, and mobilizing them for the field days. For other participants, different methods of invitation or a combination of methods (letters and e-mails) can be used. Reminders should be followed up by telephone as the date of the field day approaches. Depending upon the level of the field day, officials representing local or higher level administrations, MoA and BoA offices, representatives from research institutes, public and private seed companies, food processors, health bureaus, the media, etc. should be invited. The venue, gathering place, and time should be made known to the participants in the invitations to avoid confusion.

8.2.2 Encouraging women's participation

The QPM technology is primarily concerned with improving household food and nutrition in farming households that consume maize as their main staple and have limited access to other sources of protein. The main beneficiaries of QPM will be young children and lactating mothers. Therefore, since household nutrition traditionally is the responsibility of women, QPM promotion and adoption directly concerns women. Women's participation in QPM promotional and educational activities, especially field days, is vital. The NuME Project set a target of having at least 40% women participation in field days. To encourage women's participation, various strategies may be used, such as:

- organizing women-only field days;
- convincing husbands to come with their wives, and offering incentives (e.g., rewards, prizes, etc.) to those that do;
- using women's forums, vaccination days, and traditional social institutions like *Edir*, etc. to approach women and encourage attendance;
- organizing field days at a time that is convenient for women and does not interfere with household duties; and
- conducting food preparation demonstrations during field days.

Organizers are encouraged to develop other innovative ways of increasing women's role in dissemination activities. During discussion sessions, women who have hosted demonstrations should be encouraged to express their opinions and other women participants should also be encouraged to give their comments both on the field visit and QPM-based foods served during the field days.

8.2.3 Conducting the field days

The following is a suggested agenda for conducting field days which may be modified according to the particular circumstances:

- *Gathering of participants*: Farmers and other participants should come together at a suitable meeting place that can accommodate all participants, such as a big hall or an open field.
- *Field day program and purpose*: The facilitator of the field day introduces the program of the day and explain the objectives of the event.
- *Welcome address*: Participants are welcomed by *kebele* or *woreda* officials.
- *Official opening*: An invited official (from the region or zone or *woreda*) opens the event.
- *Pre-visit briefing*: Participants are briefed on how to visit the demonstration plots, including but not limited to: (i) the plot arrangement/layout, (ii) care must be taken during the visit to avoid breaking plants, opening ears, or taking ears, etc. Brief agronomic information including the names of the varieties, seed rate (row and plant spacing), planting date, and fertilizer rates (although usually included on plot labels) should also be provided at this time. While farmers should be encouraged to examine the crop according to their own criteria, the participants should also be briefed on which aspect of the crop to see and judge.
- *Formation of groups*: With the help of the facilitator, participants may be separated into two or three groups, depending upon the number of demonstrations plots to be visited, and are guided to the demonstration plots.
- *Field visits:* Each group is rotated from one plot to the other, allocating sufficient time at each plot for them to compare and evaluate QPM against conventional varieties; the groups then give their feedback during the discussion session. Farmers usually evaluate maize varieties based on plant height (in terms of biomass production for livestock feed and susceptibility to damage by wild animals), early maturity, especially in moisture stress areas, number and size of ears per plant (indicative of grain yield), disease resistance, and lodging tolerance. All observations, comments, and opinions during the discussions should be recorded by a responsible individual assigned to do so and subsequently summarized in a field day report.

- *Discussion session—participants' observations*: After all the demonstrations are visited by each group, the groups reconvene at the meeting place for a discussion session, which will be chaired by an invited official. Women and men farmers are asked to give their observations and opinions concerning the varieties and their characteristics.
- *Discussion session—information, questions and answers*: The facilitator or an invited professional explains what QPM is and the agronomic and nutritional benefits of the demonstrated QPM varieties. **Information and messages should be consistent with those summarized in Section 8.4 and described in detail earlier in this guidebook**. Participants are then invited and encouraged to make comments and ask questions about the QPM technology. Appropriate officials/professionals among the participants are invited to respond to the questions raised (e.g., seed company representatives will address the issue of seed availability) and also make their own comments.
- *Discussion session—QPM food preparation*: When there is a demonstration of QPM-based traditional and new foods (see Section 8.3 for details), selected women and men farmer panelists are invited to give feedback to the audience on the food they tasted.
- *Closing remarks*: The chairperson concludes the discussion, gives directions on the way forward, shares responsibilities accordingly, and closes the event or invites another official to make a closing speech.
- *Estimation of participants*: During the discussion sessions, participating men and women farmers (disaggregated) should be counted. This is best done by dividing the audience into separate groups and, depending on the number of participants, having at least two individuals, and perhaps as many as four or five, count the number of women and men participants in each group.

8.3 QPM utilization demonstration

8.3.1 Food preparation and demonstration

In addition to field demonstration of QPM varieties, traditional and new foods prepared from QPM varieties should be demonstrated during each *woreda*-level field day. Not only do food demonstrations show that dishes prepared with QPM are as good or better than those made from CM (see below), they also provide an added incentive/ attraction for women to participate in the event. To hold this demonstration, it is necessary to make sure that QPM grain or flour is available in advance. If it is not available from research centers, it may be necessary for *woreda* extension personnel or home agents associated with the Project to purchase and store sufficient high quality QPM grain from farmers who hosted QPM demos the previous season.

On the morning of the field day, while participants are visiting the demonstration plots, a range of common foods is prepared by the local people with the help of food science professionals using QPM and CM. Where BHQPY545 is being demonstrated, foods prepared from both yellow and white QPM grain should be prepared to show the different types of foods that can be prepared and to determine whether they look good to the eye. The dishes are displayed at the meeting place for all participants to see as they return from the field visit. During the food demonstration, five men and five women farmers are asked to volunteer or are randomly picked from participant farmers to taste the dishes. As indicated in Section 8.2.3, the panelists give their feedback to the field day participants.

8.3.2 Food sensory evaluation (triangle test)

To obtain a more objective and unbiased evaluation of dishes prepared with QPM compared with CM, a food sensory evaluation methodology may be used. A food sensory evaluation measures a consumer's reaction using one or more of the five senses (sight, smell, taste, touch, and hearing) to analyze and evaluate the food products. The characteristics assessed are appearance, odor, taste, texture, and sound. Sensory analysis requires the use of a panel of evaluators, and test results are recorded based on their responses to the products being tested. There are many different types of sensory tests, such as the triangle test, in which three coded samples of the same type of food are presented to each panelist. Two of the samples are identical and one is different; the panelist is asked to pick which sample he/she feels is different from the other two. The panelist should take a sip of water before tasting the food samples and between samples to clean the palate of the after-taste of the previous dish.

To avoid color bias, it is advisable to use QPM and conventional check varieties of the same color for the sensory evaluation, or have the panelists wear blindfolds so that they cannot see the preparations. Moreover, since sensory differences between QPM and CM may be disguised by spices or mixing with other ingredients (such as vegetables), at least one dish should be unadulterated maize (e.g., boiled or roasted cobs, or *injera* without sauce). The triangle test is easily adapted to the field demonstration setting, since it can be made entertaining to the audience of field day participants as they watch to see if the panelists (their friends, relatives, or neighbors), who do not know which of three identical dishes was prepared from QPM and which from CM, can identify which one is QPM and whether it is the one they felt tasted better.

When combined with field days, sensory tests should be conducted under calmer conditions (low noise level, free from foreign/odd odors, in large rooms or in the shade, far from the place where the food is prepared, etc.) to reduce panelists' bias when viewing and perceiving the product(s). Samples must be presented in random order with the label hidden (e.g., on the bottom of the dish) or with an assigned product code, such as a three-digit number (e.g., 767, 312, and 189 for three dishes) to keep food products anonymous and avoid influencing the panelists' decisions. A sensory analysis questionnaire is given to each panelist to record his/her perception of the product. The questionnaire needs to be designed in a simple, practical way to elicit clear and concise answers. Both male and female farmers and consumers should be involved in sensory evaluations of QPM-based food products in focal areas to ensure their acceptability.

8.3.3 Procedures for conducting the triangle sensory test in a field day setting

The following is a step-by-step procedure for conducting the triangle sensory test in a field day setting:

- Prepare two identical dishes at the same time, following identical procedures and measures. Prepare one of the dishes with CM and the other with QPM.
- Divide the dish prepared with CM into two portions and place each portion in a different container.
- Place an equal quantity of the QPM-based dish in another container.
- Label each container with a code; record the code of each dish with its identity on a piece of paper and give it to the chairperson for safe-keeping, to be revealed only after the test has been completed. (Alternatively, stick a label with either QPM or CM written on it on the bottom of the container, where it cannot be seen.)
- Place the three dishes on a table in front of the audience; panelists (five a time) should stand behind the table.
- Panelists are asked to turn their backs to the table and then the dishes are rearranged in random order.
- Each panelist is asked to turn to the front again while the others remain with their backs to the audience; if the dishes are of different color or appearance, he/she is blindfolded before turning around.
- The panelist is asked to taste each dish (sipping water between each) and to indicate which one is different (1st, 2nd or 3rd); the response is recorded by the chairperson against the code of the dish. Other questions (e.g., which tastes better?) may also be asked.
- When each panelist has taken his/her turn, the results are revealed by the chairperson; the results will eitherconfirm that there is no detectable difference between the QPM and CM dishes, or indicate there is a detectable difference, with either QPM or CM being preferred (or a mixed result).
- Have a discussion with panelists on relevant questions, such as: were they able to detect a difference, what sort of sensory differences (any texture, flavor, or color) were they able to perceive between the two food products, etc.

8.4 Information messages

Information and explanations about QPM technology and the nutritional benefits of the QPM varieties demonstrated must be consistent and accurate. Different information materials such as leaflets and brochures should be provided to participants. Key information messages in all QPM-related communications should include:

- Of all the cereal crops produced in Ethiopia, maize is first in terms of total production, and most people in the Ethiopian maize belt rely on maize as a source of both energy (carbohydrates) and protein. However, CM varieties are deficient in essential amino acids, specifically lysine and tryptophan.

- In areas where maize contributes more than 60% of the dietary protein intake, about 85-90% of the Ethiopian population is at risk of inadequate lysine intake. Young children are especially vulnerable.
- Unless the daily need for lysine and tryptophan is met through other lysine- and tryptophan-rich foods, relying on maize as the principal daily food causes malnutrition in humans and monogastric animals.
- Quality Protein Maize (QPM) helps to overcome the protein quality limitations of CM. Several QPM varieties have been developed and deployed by CIMMYT and its partners worldwide.
- QPM contains nearly double the amount of lysine and tryptophan available in conventional or regular maize.
- The availability and utilization of amino acids from QPM protein is 90% of that of milk.
- Agronomically, QPM looks and performs like CM, and the plant and grain can be reliably differentiated only through laboratory tests.
- QPM can reduce the risk of malnutrition and enhance nutritional security, especially among the poor and those who depend largely on maize in their daily diet and who have limited options to obtain other sources of essential amino acids.
- QPM is the product of conventional breeding and thus not a GMO (genetically modified organism).
- QPM is a cheap source of protein because farmers grow, manage, harvest, and consume it in the same way as they do CM, but with better protein quality.
- Since 2003, intensive cooperation between the EIAR and CIMMYT maize breeding programs has resulted in the commercial release of six QPM hybrids and open-pollinated varieties adapted to all the major maize-producing agro-ecologies in Ethiopia; these varieties can be readily demonstrated in relevant agro-ecologies and scaled out.
- The adoption of newly improved QPM varieties and their production practices also contributes to increased food security and higher household incomes among resource-poor smallholder Ethiopian maize farmers, as well as among farmers shifting from other staple crops due to maize's higher productivity.
- QPM grain can be used to prepare different traditional and modern foods that are very tasty.
- Women have a decisive role in household nutrition. Therefore, the strong involvement of women in QPM dissemination is more helpful in achieving household food and nutrition security.
- When growing QPM, if land is not a limitation, avoid planting CM varieties nearby.
- Store the grain harvested from QPM and CM fields separately.
- Do not mix grain harvested from QPM and CM fields for selling or in-house food preparation.

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CIMMYT-Ethiopia
Nutritious Maize for Ethiopia (NuME) Project
Tel: +251 11 617-20 00
P.O. Box 5689
Addis Ababa, Ethiopia

International Maize and Wheat Improvement Center (CIMMYT)
Apdo. Postal 6-641
Mexico D.F. Mexico 06600
Tel: +52 (55) 58042004
www.cimmyt.org