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Breeding Maize (Zea mays L.) to Improve Protein Quality in the Endosperm: A Review

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Abstract
Providing maize cultivars with an improved amino acid profile, for communities that rely heavily on maize as the main staple food is one of the main target in the modern maize breeding program worldwide. After efforts have been made by many researchers, several quality protein maize (QPM) populations and pools possessing different ecological adaptation, maturity, grain color, and texture were developed. Consumption of QPM instead of conventional maize (CM) leads to a 12% increase in the rate of growth in weight and a 9% increase in the rate of growth in height in infants and young children with mild to moderate under nutrition from populations in which maize is a significant part of the diet. The development of high lysine/tryptophan maize involves manipulating three distinct genetic systems: (1) The simple recessive allele of the *opaque-2* gene, (2) Modifiers/enhancers of the *o2o2*-containing endosperm to confer higher lysine and tryptophan and (3) Genes that modify the *opaque-2*-induced soft endosperm to hard endosperm. An appropriate application of marker assisted selection will greatly enhance the efficiency of selection for improvement of grain protein in maize besides cutting down at cost and time.
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INTRODUCTION

Maize is one of the three most important cereal crops widely grown throughout the world. The world maize production area in 2020 was around 202 million hectares, and that of wheat and paddy rice was 219 and 164.2 million hectares, respectively (FAOSTAT, 2020). Maize surpasses wheat and rice in terms of actual production. Worldwide maize production was more than 1.2 billion tonnes in 2020 - considerably more than wheat 761.0 million tonnes or rice 757.0 million tonnes (FAOSTAT, 2020). The share of Africa's maize production in 2020 was 90.5 million tonnes harvested from 43.1 million hectares. This was higher than wheat and rice, each contributing at 25.2 million tonnes from 9.97 million hectares and 37.89 million tonnes from 17.17 million hectares respectively (FAOSTAT, 2020).

Many millions of people worldwide are dependent on maize as a staple food. Maize accounts for 15 to 56% of the total daily calories of people in about 25 developing countries (Prasanna et al., 2001). In Africa, maize supplies at least one fifth of total daily calories consumed and accounts for 17 to 60% of people's total daily protein supply in 12 countries, as estimated by FAO food balance sheets (Krivanek et al., 2007). The estimated maize consumption in the African region where it is a staple food ranges from 52 g/person/day in Uganda to 328 g/person/day in Lesotho (Ranum *et al.,* 2014).

In Ethiopia maize is becoming increasingly important in terms of production and area coverage. It contributes the greatest share of production and consumption along with other major cereal crops. In 2020 main cropping season, maize was cultivated on 2.4 million hectares and resulted 10.02 million tonnes of annual production (FAOSTAT, 2020). Maize has assumed a significant importance in the diets of rural Ethiopia and gradually penetrated into urban centers. This is particularly evidenced by green maize being sold at road sides throughout the country as a hunger-breaking food available during the months of February to May annually (Twumasi *et al.,* 2012).

Despite its increased consumption largely as a source of carbohydrates, maize, like all cereal crops, is known to be of poor protein nutritional quality. The maize protein is limited in two essential amino acids - lysine and tryptophan (Bressani, 1991). Utilization of quality protein maize (QPM) varieties can correct this deficiency and may be advantageous in the diets of monogastric animals (Hai *et al.*2010). The term QPM refers to maize genotypes whose lysine and tryptophan levels in the endosperm of the kernels are about twice higher than in conventional maize (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 (Vivek et al., 2008). Therefore to develop maize varieties with high level of lysine and tryptophan, a continuous searching and identifying new and better mutants has been underway during the past several decades. Its 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 (Prasanna *et al*., 2001). The discovery of the biochemical effects of mutant alleles *o2* (Mertz et al., 1964) and *floury-2* (*fl2*) (Nelson *et al*., 1965) by the Purdue University researchers opened an exciting opportunity for

improving the quality of maize endosperm protein.

Providing those maize cultivars with an improved amino acid profile, for communities that rely heavily on maize as the main staple food is one of the main target in the modern maize breeding program worldwide. Therefore having the knowledge on methods of maize breeding for quality protein is very essential to meet the ultimate goal of maize breeding for protein quality. Thus the objective of the present paper is to review breeding methods and overview efforts done to improve the protein quality of the maize.

History of QPM Development

Quality protein maize is looks and performs like CM 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 QPMA (Adefris *et al*., 2015). The term Quality protein maize (QPM) refers to maize having *o2* gene in the homozygous recessive state (*o2o2*), high lysine and tryptophan levels, and an endosperm hard enough to ensure acceptable ear characteristics (Vivek *et al.*, 2008)

Several natural maize mutants conferring higher lysine and tryptophan were identified in the 1960s and 1970s, viz., *opaque-2* (*o2*), *floury-2* (*fl2*), *opaque-7* (*o7*), *opaque-6* (*o6*), and *floury-3*(*fl3*) (Table 1) (Vivek *et al.*, 2008). 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 (Adefris *et al*., 2015). Soon after, 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 (Prasanna et al., 2001).

Table 1: High lysine mutants of maize

Gene	Allele	Researchers	Year of discovery
Opaque-2	o2	Mertz, Bates and Nelson	1964
Floury-2	fl2	Nelson, Mertz and Bates	1965
Opaque-6	o6	McWhirter	1971
Opaque-7	o7	Ma and Nelson	1975
Floury-3	fl3	Ma and Nelson	1975

Source: Vivek *et al.*, 2008

The effects of those maize mutants include reduced grain yield (as compared to normal maize), low kernel density, soft and chalky kernel phenotype, greater vulnerability to ear rot, greater moisture content during dry-down of kernels following physiological maturity, lower rate of germination and greater kernel breakage (Vasal, 2000, Prasanna et al., 2001). 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.

After efforts have been made by many researchers, 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. 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. 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) (Babu and Prasanna, 2014).

The QPM development program in Ethiopia was launched in 1994 with the evaluation of open-pollinated varieties (OPVs) and pools introduced from CIMMYT (Leta *et al*., 2001). Then quality protein maize development program was initiated for the highland, mid-altitude, and moisture-stressed maize agro-ecologies of Ethiopia, with emphasis on (1) Screening QPM varieties introduced from elsewhere for adaptation to local conditions,(2) Conversion of popular and farmer-preferred CM cultivars into QPM versions. (3) QPM source germplasm development through mass conversion of elite non-QPM inbred lines or pedigree breeding with proven QPM lines (Adefris *et al*., 2015). 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 (Adefris *et al*., 2015).

Nutritional Benefits of QPM

The Nutritional benefits of quality protein maize grain, due to its high levels of the essential amino acids lysine and tryptophan, has been extensively studied (Akalu *et al*., 2010, Gunaratna *et al*., 2010). 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. The nutritional benefits, especially for people who depend on maize for their energy, protein, and other nutrients, are sufficient to justify its wide scale production and promotion.

Study on *o2* maize (Bressani, 1992) 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 and found that a greater proportion of protein from *o2* maize, compared with conventional maize, is available for utilization by the body for normal physiological processes and growth. Consumption of QPM instead of CM leads to a 12% increase in the rate of growth in weight and a 9% increase in the rate of growth in height in infants and young children with mild to moderate under nutrition from populations in which maize is a significant part of the diet (Gunaratna *et al*., 2010). As in the case of infants and children, QPM had equally beneficial effects on adults (Bressani, 1992).

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 (De Groote *et al*., 2014). A community based study conducted in the eastern Wollega Zone of Ethiopia by the Ethiopian Health and Nutrition Research Institute (EHNRI) showed that in areas where maize is cultivated mainly for consumption, use of QPM in children's diets could enhance growth and development. In this study, growth was monitored in 160 young children of maize-producing families (Akalu *et al*., 2010). They 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.

Apart from the key roles in human health, the nutritional and biological superiority of QPM has also been amply demonstrated in animals. Pigs raised on high lysine/tryptophan maize gain weight at roughly twice the rate of animals fed solely on normal maize with no additional protein supplements. An equal quantity of high lysine maize substituted for normal maize in pig feeds can maintain the amino acid balance and decrease the use of synthetic lysine (Burgoon et al., 1992). The nutritional advantage of QPM over CM was also demonstrated in weaner pigs (Mpofu *et al*., 2012). QPM also provides a cheaper way of obtaining a balanced animal feed and fattening with excellent monetary returns (Krivanek *et al*., 2007). 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). Overall, QPM possess superior protein quality and enhances food and feed efficiency.

Biochemical Characteristics

Cereals provide energy, protein, fiber, vitamins and minerals. Protein composition and content are particularly important, not only in terms of nutrition, but also the impact on quality of targeted end-uses of cereals (Shewry & Halford, 2002). Cereal proteins are classified into the following four groups based on their solubility (Singh, 2005): (1) albumins (water soluble), (2) globulins (salt soluble), (3) prolamins (relatively highly alcohol soluble) and (4) glutelins (dilute alkali soluble). In normal maize endosperm, the proportions of various protein fractions on average are albumins 3%, globulins 3%, prolamines 60%, and glutelin 34% (Schnieder, 1955). For most cereals, prolamins are given names based on their generic Latin names (Shewry & Tatham, 1990). For maize, they are known as zein (from *Zea mays*), barley as hordein (from *Hordeum vulgare*) and rye as secalin (from *Secale cereale*).

The zeins can account for 40-60% of the total protein in the maize endosperm, and, because of their abundance, they are the primary determinants of the amino acid composition in maize kernels (Singh, 2005). Zeins are a class of alcohol soluble proteins that are specific to endosperm of maize (Prassana et al., 2001) and are not detected in any other plant part. Zeins consist of one major class (α-zeins) and three minor classes (β, γ and δ). The zein fraction in normal maize normally contains higher proportion of leucine (18.7%), phenylalanine (5.2%) isoleucine (3.8%), valine (3.6%) and tyrosine (3.5%), but smaller amounts of other essential amino acids such as threonine (3%), histidine and cysteine (1%), methionine (0.9%), lysine (0.1%) and is essentially devoid of tryptophan as it is absent from the major prolamin fraction (α-zeins) of maize kernel (Sofi *et al*., 2009). The non-zein protein fraction is balanced and rich in lysine and tryptophan (Vasal, 2000).

Darrigues et al. (2006) illustrated that in the amino acid balance of maize, lysine and tryptophan are the most deficient; histidine and leucine are surplus amino acids as compared to the egg protein which is a nearly balanced source of protein. Therefore introduction of high quality protein maize mutants alters the relative amounts of four major protein fractions present in maize (Darrigues et al., 2006). Kernels carrying homozygous *o2* mutant have increased the levels of lysine and tryptophan by reducing the synthesis of the lysine-deficient zein fraction (Habben et al., 1993). Since fractions other than zein are higher in lysine and tryptophan, zein

reduction causes proportional elevating of other fractions high in lysine (Habben et al., 1993; Vasal, 2000). The result is that the levels of lysine and tryptophan become raised in protein, but not on absolute basis of per unit endosperm (Vasal, 2001). Therefore, increasing the levels of lysine and tryptophan should be important goals for maize breeding efforts directed to improving grain amino acid balance.

Genetics of High Lysine and Tryptophan Maize

The development of high lysine/tryptophan maize involves manipulating three distinct genetic systems (Krivaneket al., 2007):

1. The simple recessive allele of the *opaque-2* gene,
2. Modifiers/enhancers of the *o2o2*-containing endosperm to confer higher lysine and tryptophan,
3. Genes that modify the *opaque-2*-induced soft endosperm to hard endosperm.

1. The Simple Recessive Allele 0f the *Opaque-2* Gene

Jones and Singleton first described the opaque-2 (o_2) mutation in the early 1920s but Mertz and coworkers discovered the nutritional significance of the opaue-2 mutation (Prasanna *et al*., 2001). This is a central component of the genetic system that confers higher levels of lysine and tryptophan in maize endosperm protein (Vivek *et al*., 2008). The *o2* allele is inherited in a simple recessive manner (Figure 1). The presence of *o2* in the homozygous recessive (*o2o2*) state is a pre-requisite for the entire process of obtaining high lysine/tryptophan maize, discussed in the following sections. The most abundant proteins in the grain endosperm are the zeins and, particularly, α-zein, which are poor in lysine and tryptophan (Gibbon and Larkins, 2005).

The homozygous *o2* mutant causes a decrease in the production of alpha-zein fraction of endosperm protein and a corresponding increase in the proportion of non-zein proteins (Albumins, Glubulins, Glutelinlike and Glutelin) that naturally contain higher levels of lysine and tryptophan (Gibbon and Larkins, 2005). Therefore, in a given quantity of protein from o2o2 maize, the proportion of non-zeins is higher, which predisposes *o2* maize to have higher lysine and tryptophan. However, the presence of the *o2* allele in the recessive condition (*o2o2*) alone does not ensure high lysine and tryptophan levels, but only predisposes maize to have them. The presence of another set of modifiers/enhancers of the *o2o2*-containing endosperm genes that enhance the levels of lysine and tryptophan is required to confer higher levels of these amino acids.

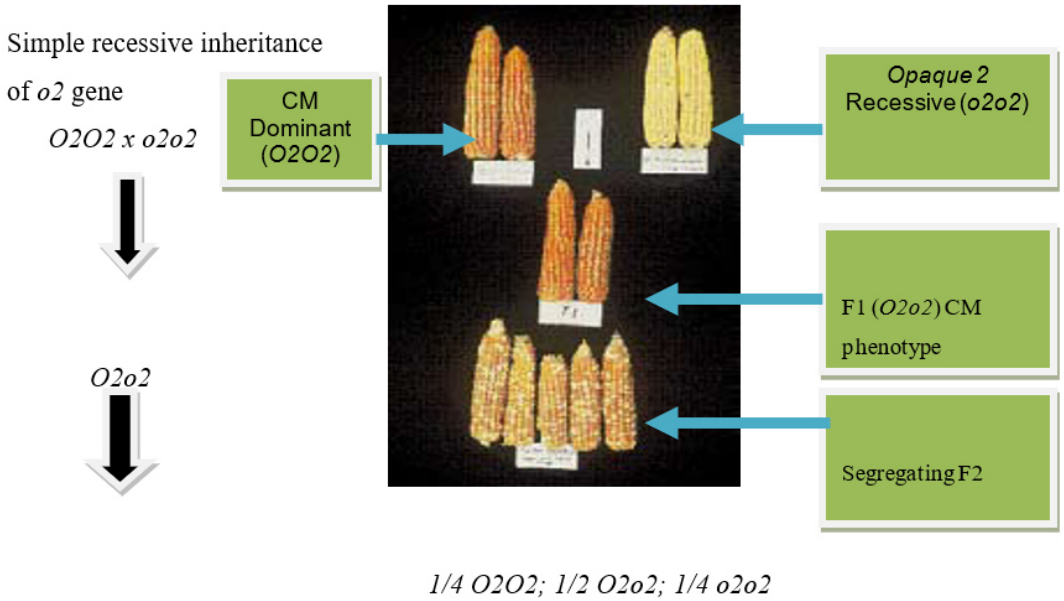

Figure 1: Simple recessive inheritance of the *o2* gene source:- Vivek *et al.*, 2008

2. Modifiers/Enhancers of the *o2o2*-Containing Endosperm to Confer Higher Lysine and Tryptophan

Opaque-2 is a mutation in one of the regulatory loci that control storage protein genetic transcription in maize kernels. This is the second essential genetic system that confers higher lysine/tryptophan in maize. They cause a reduction in a storage protein (zein or prolamins) content of the endosperm and a significant increase in protein fraction of non zeins, which is nutritionally more balanced. It consists of minor modifying loci that affect lysine and tryptophan levels in the endosperm. Lysine levels in normal and *o2* maize average 2.0% and 4.0%, respectively, of total protein in whole grain flour. However, across diverse genetic backgrounds, these levels range from 1.5-2.8% in normal maize to 2.6 - 5.0% in their *o2* converted counterparts (Moro *et al*., 1996). Therefore, if lysine or tryptophan levels are not monitored while developing new cultivars, one could end up with a maize cultivar having the *o2o2* genotype and lysine and tryptophan levels equivalent to those in normal maize, since the lower limits of lysine and tryptophan in *o2o2* maize overlap with the upper limits in normal

maize (Table 2). Wallace *et al.* (1990), shows that an increased level of gamma zein likely contributes to the recovery of a hard endosperm phenotype, given that the *o2*-modified (hard endosperm) grains have approximately double the amount of gamma zein in the endosperm as the *o2*-only mutants. (While the proportion of zeins generally decreases in *o2* germplasm, gamma zein increases during the recovery of hard endosperm. The beneficial alleles of the modifying loci that control gamma zein production can be selected using a rapid, low-cost, and light-table method (Vivek *et al*., 2008).

Table 2: Lysine and tryptophan levels as percentages of total protein in whole grain flour of normal and *o2o2* maize.

	Normal maize	o2o2 maize
Lysine	1.6-2.6 (mean 2.0)	2.7-4.5 (mean 4.0)
Tryptophan	0.2-0.6 (mean 0.4)	0.5-1.1 (mean 0.8)

Source: (Vivek *et al*., 2008).

3. Genes That Modify the *Opaque-2*-Induced Soft Endosperm to Hard Endosperm

The *o2* mutation and the modifiers/enhancers of lysine and tryptophan are, by themselves, not sufficient to develop economically acceptable maize high in lysine and tryptophan. Pleiotropic effects of the *o2* allele make the maize endosperm soft and susceptible to cracking, ear rots, and weevils (Figure 2). Such negative secondary effects are obviously undesirable.

Figure 2. Soft endosperm *o2* ears showing splitting of pericarp**.**

This softness expresses itself as an opaque phenotype that can be viewed on a light table. Therefore, breeding maize for high lysine and tryptophan requires selection based on a third, distinct genetic system, also comprised of minor modifying loci that convert the mutant endosperm of the soft/opaque/floury phenotype to a hard/vitreous phenotype similar to normal maize (Vivek *et al.,* 2008).

Breeding Quality Protein Maize

Current QPM breeding strategies 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 (Adefris *et al*., 2015). Once a breeding program has some elite QPM germplasm, one could start recycling elite-QPM germplasm with elite-QPM germplasm available in the program or from other breeding programs (i.e., use elite QPM x elite QPM crosses). Whichever of the above breeding methods is chosen, there are two possible approaches to QPM breeding: the conventional approach and an approach that uses molecular markers to assist in *o2* selection.

In both breeding approach there are two unique (i.e., different from steps involved in breeding for other maize traits) and essential steps in the development of QPM germplasm: (1) Identification of segregants in a family or population having the *o2* allele in the homozygous recessive (*o2o2*) condition with a hard endosperm (identified simultaneously). The conventional approach involves using a light table while, in the molecular approach, leaf samples of candidate plants are analyzed using markers to identify the *o2o2* genotype, but, as in the conventional approach, a light table is needed to differentiate hard endosperm types from the *o2o2* genotypes. (2) Identification and confirmation of protein quality (percentage of tryptophan and protein in sample) through laboratory analyses (Vivek *et al.,* 2008).

Tools for Identification of QPM from Non QPM Kernel

1. Light Table

A light table is a custom-made box used to differentiate hard endosperm maize types from soft *o2o2* genotypes.

It is usually made of wood on all sides, except for the top surface, which is made of semi-transparent glass or plastic. Inside the box, one or more fluorescent (or other type) bulbs are placed in a lamp connected to an outside power source. The kernel characteristic is viewed, placing maize grain on the table and turning on the light. Different sizes of light tables are used starting from the minim desirable size (27.5cm long x 15 cm wide x 7.5 cm high (Vivek *et al.,* 2008). Holes on two opposing sides of the box provide adequate ventilation and prevent overheating.

Light table selection is based on the principle that *o2o2* genotypes carry an undesirable characteristic, kernel softness, which, on a light table, is seen as complete opaqueness. Due to segregation of genes for endosperm hardness (or softness), varying degrees of softness/hardness are expressed in the endosperm of segregating generations (i.e., varying levels of opaqueness are observed on a light table. A kernel with the *O2o2* or *O2O2* genotype is normal maize, i.e., it does not have the softness and undesirable kernel characteristics associated with the *o2o2* genotype and is therefore translucent. Light tabling is done to pick out kernels with the *o2o2* genotype by using the degree of opaqueness as an indirect measure or secondary trait. Gradation in the opaqueness is scored from 1 to 5, where 1 = completely modified (that is translucent normal phenotype); 2 = 75% modified; 3 = 50% modified; 4 = 25% modified; and 5 = completely opaque (Vivek et al.,2008) as shown in Figure3.

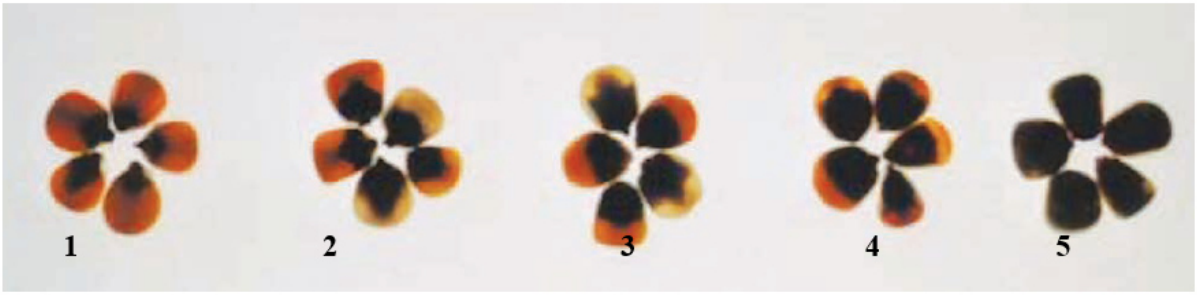

Figure 3: Varying degrees of opaqueness indicate varying levels of endosperm modification: 1 = 100% modified; 2 = 75% modified; 3 = 50% modified; 4 = 25% modified; and 5 = completely opaque Source: Kassahun and Prasanna (2004)

When working with the conventional breeding approach light table selection is done on all segregating generations. It is especially important in early (F2 to F6) generations of inbred line development. Modifiers are a set of minor genes. Hence kernels selected for type 3 in an early generation (F_2) will very likely produce a whole range of kernel types in the next generation due to segregation of minor genes. Homozygosity increases with successive generations of inbreeding. As modification type 2 is progressively selected, one moves towards fixation of this modification level in the kernel. Elite QPM inbred lines ideally have the modifiers fixed at a score of 2, but it is not uncommon to find elite inbred lines with a modification score of 3 (Vivek *et al.,* 2008).

2. Protein Quality Laboratory

Samples are usually first sent to the laboratory for protein content and tryptophan analysis at the F3 or F4 stage (*Vivek et al.,* 2008). This helps us to undergo laboratory analyses, both lysine and tryptophan concentrations are increased in QPM materials. These increases need to be monitored during the breeding process, but only tryptophan is analyzed on a routine basis. This is because lysine (Lys) and tryptophan (Trp) values are highly correlated (Hernandez and Bates, 1969). Normally, the value of lysine is four times that of tryptophan. Due to the well-established relationship between these amino acids in the protein of *opaque-2* maize endosperm (*Villegas et al.,* 1992), tryptophan can be used as a single parameter for evaluating the nutritional quality of the protein.

Marker-Assisted Selection (MAS) For QPM Breeding

Marker-assisted selection (MAS) is a procedure that has been developed to avoid problems associated with phenotypic selection, replacing the selection of the phenotype by selection of genes, both directly and indirectly (Francia *et al*. 2005). The development of QPM requires manipulation of various genetic systems such as *o*2, endosperm modifiers and amino acid modifiers and as such conventional breeding procedures are quite laborious and the results sometimes frustrating (Sofi *et al*., 2009). It is very tedious to continuously select for optimum level of one trait while maintaining desired level of other. Besides, low cost and reliable methods of screening are not available. MAS is an appropriate technology for traits such as high lysine in maize and can be a cost effective procedure for selecting *o2* locus in breeding populations (Dreher et al., 2003). With sequencing of maize genome being finished, a large number of market system are now available that are associated with *o2* and endosperm modification phenotype (Lopez et al., 2004; Bantle and Prasanna, 2003).

An appropriate application of such markers will greatly enhance the efficiency of selection for improvement of grain protein in maize besides cutting down at cost and time (Sofi *et al*., 2009). Babu et al. (2005) used MAS

for development of QPM parental lines of Vivek-9 hybrid and could developed QPM hybrid in less than half the time required through conventional breeding. Danson et al. (2006) used various markers to introgress *o2* gene into herbicide tolerant elite maize inbred lines. They found that using marker for QPM and endosperm modification in tonden can greatly enhance the selection efficiency for isolating fully modified kernels in QPM background.

Recently Babu and Prasanna, (2014) also reported that 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 single nucleotide polymorphism 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

CONCLUSION

Biochemical analysis is required to determine the lysine and tryptophan content of the seed and confirm whether or not it is QPM. QPM developed from mutant maize, contains nearly twice the amount of Lysine and Tryptophan amino acids. The higher lysine and tryptophan contents of QPM varieties, compared to CM, provide a more balanced protein for humans and other monogastric animals. The development of high lysine/tryptophan maize involves manipulating three distinct genetic systems (1) The simple recessive allele of the *opaque-2* gene, (2) Modifiers/enhancers of the *o2o2*-containing endosperm to confer higher lysine and tryptophan and (3) Genes that modify the *opaque-2*-induced soft endosperm to hard endosperm. Development of QPM germplasm involves (1) Identification of segregants in a family or population having the *o2* allele in the homozygous recessive (*o2o2*) condition with a hard endosperm and (2) Identification and confirmation of protein quality (percentage of tryptophan and protein in sample) through laboratory analyses. An appropriate application of MAS will greatly enhance the efficiency of selection for improvement of grain protein in maize besides cutting down at cost and time.

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