Chapter 7
Genetics and Applied Genomics of Quality Protein Maize for Food and Nutritional Security

P. K. Agrawal, M. G. Mallikarjuna, and H. S. Gupta

Abstract Maize (*Zea mays* L.) is an important food and feed crop of the world. Together with rice and wheat, it provides around 40% of the food calories to more than 4.5 billion people in 94 developing countries. It also provides nearly 50% of the dietary protein for humans. In Africa and some of the Asian countries, almost 90% of maize grown is for human consumption and may account for 80–90% of the energy intake. In India, it is the third most important food crop after rice and wheat, both in terms of area and production. India is the fifth largest producer of maize in the world contributing 3% of the total global production. Protein malnutrition is widespread in the developing and underdeveloped countries, where 780 million people are affected by the same. Maize is the leading cereal in terms of production and accounts for 15% of proteins and 20% of calories requirement of the world. Protein malnutrition is caused by lack of access to adequate quantity and better quality protein intake and usually affects children and elderly persons. Maize, however, lacks adequate amounts of the essential amino acids, namely, lysine and tryptophan. Decades of efforts by maize researchers lead to the development of nutritionally superior maize cultivar popularly called as quality protein maize (QPM), which has twice the amount of lysine and tryptophan, thus making its quality as good as casein of milk. The *o*2 allele along with modifiers for tryptophan and lysine content and grain hardness made QPM agronomically suitable for cultivations. Intensive efforts were made by many workers to understand the genetics, molecular mechanism of QPM modifiers and applied these genomics knowledge to developed MAS-based QPM inbreds and commercial hybrids. All those studies and concerted efforts led to development and utilization of QPM. The area under QPM globally is more than 9.0 million hectares. Several reports were available on positive impact of QPM on children and adults. It has also been demonstrated in poultry and

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piggery, resulting in increased egg production and egg quality parameters and body mass. The area under QPM and consumption of QPM can be increased significantly by providing policy supports for QPM.

Keywords Lysine · Malnutrition · MAS · Maize · Nutrition · Policy · Protein · QPM

7.1 Introduction

Tremendous advances in agricultural sciences have although helped in enhancing food production remarkably; yet, malnutrition continues to be a worldwide problem especially in the developing and underdeveloped countries. Globally, 795 million people are chronically undernourished, and, out of this, nearly 780 million live in developing world (https://www.worldhunger.org). The problem is more severe in preschool children and women. In developing countries, about 32% of preschool children are stunted, and 20% are underweight due to protein malnutrition alone (Black et al. 2008). Cereals are the major contributors to global food security with an annual production of ~2.3 billion tonnes. Out of it, nearly one billion tonnes is used for human consumption, 750 million tonnes for animal feed, and the remaining 500 million tonnes for industrial use as well as for seeds and other purposes (FAO 2015; Shewry 2007). Thus, cereals contribute more than 50% of the dietary protein requirement globally and 70% in developing countries (Gibbon and Larkins 2005). Among cereals, maize, wheat, and rice contribute over 85% of total cereal production and accounts 42.5% of world calorie requirement (Shiferaw et al. 2011; FAO 2015). However, in most of the developing nations where single multi-cereal-based diet predominates, the nutritional profile of such cereal(s) assume great significance.

Maize leads the global cereal production with an annual output of 1060 million tonnes and contributes more than 100 million tonnes of proteins (assuming 10% protein) during the year 2016–2017 (http://www.fao.org/faostat/). It is the major source of daily diet in Sub-Saharan Africa, Latin America, and Asia. Although, normal maize is a good source of basic dietary requirements, it cannot provide all the essential amino acids, which are not manufactured in human body (Nelson 1969; Gupta et al. 2013). Approximately 70% of the maize protein is composed of alcohol-soluble prolamins known as zeins, namely, α, β, γ, and δ (Gupta et al. 2009). These protein fractions are rich in glutamine, leucine, and proline and distributed in a distinctive pattern in grain (Gibbon and Larkins 2005). Zeins in normal maize possess unbalanced amino acid composition with reduced concentration of essential amino acids, namely, lysine and tryptophan (Gupta et al. 2009). Reduced quantity of essential amino acids in zeins of normal maize protein brings down the biological value of maize protein to 40% of milk protein. This makes maize a poor source of protein. Supplementation of protein sources from legumes and animal products is often not viable because of cost factor. Several natural mutants with higher lysine and tryptophan, namely, *opaque-2* (*o2*), *floury*-2 (*fl2*), *opaque*-7 (*o7*), *opaque*-6 (*o6*), and *floury-3* (*fl3*), were identified in maize (Ignjatovic-Micic et al. 2008).

Among these mutants, *o2* mutant was found to be most amenable for genetic manipulation of lysine and tryptophan owing to its ability to accumulate higher lysine and tryptophan content (Bressani 1992) without reducing or altering the total protein concentration. Further, the increased concentration of lysine and tryptophan in *o2* mutants increased the biological value of maize protein to 90% of the milk protein (Bressani 1992). However, the desirable nutritive value of *o2* mutants was found to be associated with undesirable agronomic traits such as dull and chalky grains and lower grain weight and susceptibility to several diseases and insect pests. Painstaking efforts at CIMMYT, Mexico, by Vasal and Villegas led to breaking of undesirable linkage between the nutritive value and agronomically undesirable characters. Further, *o2* in combination with modifiers lead to the development of quality protein maize (QPM) (Vasal 2000, 2001, 2002). The QPM, thus, overcame all the defects associated with *o2*: soft to harder endosperm, higher yield potential, and resistance to pests and diseases. The nutritional impact of quality protein maize was well demonstrated by several international programs in Africa and Latin American countries (https://www.povertyactionlab.org).

7.2 Maize Endosperm and Its Mutants

A typical maize kernel is characterized by an endosperm and embryo. The endosperm is surrounded by an outermost aleuron layer; the starchy endosperm cells and protein are tightly packed just beneath the aleuron layer. Packing density of endosperm cells gives a typical vitreous (glassy) or starchy appearance to maize kernel. Generally, the endosperm is composed of around 90% of starch and 10% protein. The maize endosperm proteins can be classified into four types: (i) water-soluble albumins (3%), (ii) salt-soluble globulins (3%), (iii) alkali-soluble glutelins (34%), and (iv) alcohol-soluble prolamins (zeins) (60–70%) (Vasal 2000; Gibbon and Larkins 2005). Further, zeins are specific to maize endosperm (Prasanna et al. 2001). The zeins are characterized into one major class called α-zeins (19 and 22 kDa) and three minor classes, namely, β- (15 kDa), γ- (16, 27, and 50 kDa), and δ-zeins (10 and 18 kDa), and are encoded by different classes of structural genes (Coleman and Larkins 1999). The zeins are known to be very poor in lysine content (0.01%) compared to higher concentration (>2 g/100 g protein) in case of albumins, globulins, and glutelins. On the contrary, embryo protein is dominated by the albumin fractions (>60%) which are superior in terms of amino acid composition and nutritional quality (Vasal 2000). The presence of higher amount of zeins with negligible concentration of lysine in maize endosperm imparts negative effects on growth of animals (Osborne and Mendel 1914). In order to enhance the concentration of lysine in zein proteins, several high-lysine mutants were identified and characterized to overcome the limitations of zeins. *Opaque* mutants with 50% reduced zein fraction and increased concentration of nutritionally superior non-zein endosperm proteins were identified and successfully employed in the breeding program (Gupta et al. 2013; Babu and Prasanna 2014).

7.2.1 Genetics of Endosperm Mutants

A large number of maize endosperm mutants, causing modification of the endosperm and its constituents, have been reported (Thompson and Larkin 1994; Hunter et al. 2002). All the opaque mutants, namely, *o1* (Emerson et al. 1935), *o2* (Mertz et al. 1964), *o5* (Gibbon and Larkins 2005), *o6* (Ma and Nelson 1975), *o7* (Misra et al. 1972; Burr and Burr 1982), *o9–11* (Nelson 1981), and *o13–17* (Dannenhoffer et al. 1995; Gibbon and Larkins 2005; Yang et al. 2005) were recessive in nature, whereas the floury mutants, namely, *fl1* (Emerson et al. 1935), *fl2* (Nelson et al. 1965), and *fl3* (Ma and Nelson 1975) are semidominant in nature. The mutants, mucronate (*Mc*) (Salamini et al. 1983) and *Defective endosperm B30* (*De-B30*) (Salamini et al. 1979), showed dominant inheritance. In addition to simple inheritance, some of the genes show epistatic interactions and dosage effects. The *o2* and *o7* genes were reported to be epistatic over *fl2*, whereas synergetic effects were observed between *o2* and *Mc* and *o2* and *o16* (Prasanna and Sarkar 1991; Zhang et al. 2010, 2013). The dosage effects were reported for mutations *De-B30*, *fl2*, and *fl3*, where the kernel opacity and protein quality depend on the dosage of the recessive alleles in the triploid endosperm (Soave et al. 1981; Vasal 2002).

Furthermore, spontaneous mutant *o7* isolated from W22 was found located on chromosome 10 (Tsai and Dalby 1974). The *o7* possess starchy endosperm at maturity, reduced endosperm weight, and total protein content (Di Fonzo et al. 1979). Like other opaque mutants, *o*7 also inhibits the synthesis of all zein classes of proteins (Misra et al. 1972; Lee et al. 1976; Hartings et al. 2011). A new endosperm mutant *o16* was identified by Yang et al. (2005) and positioned on long arm of chromosome 8. The *o16* showed increased lysine content (~0.36%) and was found to be linked to the molecular marker umc1141. Unlike other opaque mutants, endosperm phenotype of *o5* mutant is caused by reduction of galactolipids of the endosperm without any alteration in the zeins content (Myers et al. 2011). On the other hand, floury mutants share many of the common features of opaque mutants such as reduced zein fraction, soft chalky endosperm texture, and inability to accumulate sufficient dry matter (Vasal 2002). The *fl2* mutation is caused by the accumulation of the improperly processed of 24 kDa α-zein precursor protein, and the resultant protein bodies are not only smaller than normal, but they are also asymmetrical and misshapen (Coleman et al. 1997). However, the *fl1* mutation changes the location of 22 kDa α-zeins within the protein body and does not affect the amount and composition of zeins per se (Holding et al. 2010). The other group of mutants such as *fl4*, *De-B30*, and *Mc* are associated with improper distribution of zeins and protein body deformation like *fl2* mutation (Coleman et al. 1995; Kim et al. 2006; Wang et al. 2011, 2014).

Although endosperm mutant *o2* was reported as early as in the 1920s, the nutritional significance was first showed by Mertz and co-workers in the 1960s (Singleton 1939; Mertz et al. 1964; Nelson et al. 1965). Thereafter, it has extensively been used and incorporated in a large number of maize lines; however, due to pleiotropic effect on the endosperm in terms of kernel softness, disease susceptibility, short storage time, etc., it could not become popular.

7.2.2 *Molecular Mechanism Underlying Endosperm Modifications*

7.2.2.1 Opaque Mutants

The discovery of *o2* mutant of maize in 1960s aroused great interest as it helped in enhancing biological value of maize protein by increasing lysine and tryptophan-the two essential amino acids that are not synthesized in human as well as livestocks. The *o2* mutant was most extensively studied owing to its widespread usage in the successful development of global QPM development programs. The wild allele *O2* codes for defective basic domain-leucine zipper transcription factor and regulates expression of the 22 kDa α-zeins and other endosperm-specific genes (Schmidt et al. 1987, 1990; Damerval and De Vienne 1993; Habben et al. 1993). The defective allele *o2* represses the expression of 22 kDa α-zeins genes and thereby reduces the production of zeins fraction (Schmidt et al. 1987, 1990). Unlike *o2*, the other mutations *fl2*, *fl4*, and *De-B30* encode for defective signal peptides and result in disrupted zein deposition. In *fl2* the point mutation (alanine, valine) at 22 kD α-zein signal peptide affects the processing and removal of signal peptide from mature protein. Similarly, *fl4* and *De-B30* are resulted from mutations that cause the signal peptides to remain attached with the 19 kD α-zein, resulting in aggregation of these proteins in the endoplasmic reticulum (Gillikin et al. 1997; Wang et al. 2014). In contrast to mutations at signal peptides, *Mc* gene results from a 38 bp deletion and leads to a frameshift mutation in the 16 kD γ-zein (Gibbon and Larkins 2005; Kim et al. 2006).

7.2.2.2 Endosperm Modifiers

Considerable information is available on molecular and biochemical basis of kernel texture modification in QPM. Proteomics of QPM lines showed increased granule-bound starch synthase I in the soluble non-zein fraction and resulted in shorter amylopectin branches and increased starch grain swelling (Gibbon et al. 2003). *o2* modifiers are semidominant genes and express in both *o2* and normal genetic backgrounds; however, higher expression rate during seed development was observed in modified opaque lines. The increased modifier gene expression appears to be a consequence of enhanced mRNA transcription or transcript stability (Geetha et al. 1991). The increased expression rate of *γ-zein A* plays important role in kernel opaqueness. The expression rate of γ-zein A/γ-zein B after 18 days of pollination in modified opaque-2 was 40:1 as compared to 1:1 in wild-type and 3:1 in *o2* genotypes (Burnett and Larkins 1999). In addition to *o2*, prolamine-box binding factor (PBF) regulates zein and starch synthesis. The *o2* and *PbfRNAi* showed reduced starch synthesis ~5% and ~11%, respectively. Whereas, double-mutant *PbfRNAi-o2* showed reduced starch synthesis by 25%. Transcriptome assay revealed that the expression >1000 genes related to sugar and protein metabolism was affected in *PbfRNAi*, *o2*, and *PbfRNAi-o2* mutants (Zhang et al. 2016).

7.3 Development of Quality Protein Maize (QPM)

The untiring efforts by Vasal and co-workers at CIMMYT during the 1970s and 1980s helped in overcoming the major drawbacks of *o2* mutants, namely, soft endosperm, shorter shelf life, disease susceptibility, and low yield, when high-lysine mutants *o2* was combined with modifiers. Whereas the one set of modifier genes helped in overcoming softness of the endosperm and gave rise in vitreous and hard endosperm, the other set of modifiers helped in increasing lysine and tryptophan content (Tandzi et al. 2017). Thus, the unique combination of *o2* and endosperm hardness led to the development of quality protein maize (QPM) (Vasal et al. 1980). The discovery of endosperm modifiers or *opaque modifiers* (*Opm*) revolutionized QPM breeding (Villegas et al. 1992). The endosperm modifiers possess multiple loci and complex phenotypic effects (Vasal et al. 1980; Belousov 1987; Geevers and Lake 1992). Genetic analysis of endosperm modifiers identified several quantitative trait loci (QTLs) in various source of populations. QTLs associated with *o2* endosperm modification in the QPM backgrounds of Pool 33 (CIMMYT in Mexico; Vasal et al. 1980; Bjarnason and Vasal 1992) and K0326Y (South Africa; Geevers and Lake 1992) were mapped. Pool 33 and K0326Y were crossed with a starchy endosperm Midwestern inbred W64Ao2. QTLs for *Opms* were identified in F_2-F_3 progenies segregating for a vitreous or starchy kernel phenotype by bulked segregant analysis. Three *Opms* QTLs for Pool 33 (bins, 6.03, 6.04, and 7.02) and three QTLs for K0326Y (bins, 7.02, 9.03, and 9.05) were identified. Additional seven QTLs explaining 75% of phenotypic variation were detected in the F_2 population (K0326Y QPM x W64Ao2). The bins 7.02 and 9.04/9.05 F_2 corresponded with the QTLs identified by BSA explained 40% of the phenotypic variation (Holding et al. 2008). The validation and characterization of identified QTLs for *Opms* were carried by Holding and co-workers in 2011. Genetic linkage analysis of the RIL (K0326Y QPM × W64Ao2) population validated the three of the previously identified QTLs associated with *o2* endosperm modification. The QTL located on chromosome 7 (umc1036-γ-zein) explained maximum phenotypic variation for vitreousness (38%), breakage mean (22.7%), and density (37.8%) (Holding et al. 2011). Five QTLs falling on chromosome number 5, 7, and 9 and explained 38.6% of the total phenotypic variance for *Opms* were identified in the $F_{2:3}$ population developed from hill-adopted Indian maize genotypes (VQL2 × VQL8) (Babu et al. 2015). The identified major QTLs at bin 7.02, which are candidates for *Opm* genes, showed increased 27 kDa γ-zein gene expression (Holding et al. 2008). However, the QTLs linked to *o15* showed decreased 27 kDa γ-zein expression (Dannenhoffer et al. 1995). The γ-RNAi knockdown of 27 and 16 kDa γ-zeins showed only partial opacity by an incomplete embedding of starch granules in the vitreous area (Wu et al. 2010).

Although several QTLs were identified and co-located candidate genes were characterized, the molecular mechanism by which *Opm* creates vitreous phenotype is not understood clearly. Biochemical characterization of QPM genotypes revealed

the association between large amount of the cysteine-rich 27 kDa γ-zein storage protein and dosage of *Opm* genes (Ortega and Bates 1983; Wallace et al. 1990; Geetha et al. 1991; Lopes and Larkins 1991). Furthermore, there is a correlation between γ-zein content and the number of protein bodies in the endosperm, as verified by the *o15* mutant, which has a reduced level of γ-zein and contains half of the normal number of protein bodies. γ-zein ability to form covalent linkage with other cysteine-rich proteins that leads to the formation of a tightly linked proteinaceous network surrounding the starch grains could be a reason for its association with a number of protein bodies (Dannenhoffer et al. 1995). Yuan et al. (2014) demonstrated that deletions in γ-zein caused intermediate 27 and 50 kDa γ-zein levels and were semivitreous owing to indicating haplo-insufficiency of these gene products in *o2* endosperm modification. The γ-zein as an *o2* modifier gene within the largest QPM quantitative trait locus may suggest the 50 kD γ-zein also contributes to endosperm modification. Recently, Liu et al. (2016) identified a quantitative trait locus (qγ27) affecting the expression of 27 kDa γ-zein in the same region as the major *o2* modifier loci on chromosome 7. The qγ27 resulted from a 15.26-kb duplication at the 27 kDa γ-zein locus. Although duplication might have occurred before maize domestication, the gene structure of qγ27 appears to be unstable, and the DNA rearrangement frequently occurs at this locus owing to enhanced response for artificial selection.

7.4 Breeding QPM Varieties and Hybrids

7.4.1 Conventional Breeding

The development of composites and hybrid cultivars is the major targets in cross-pollinated crops like maize. The synthesis of QPM hybrids necessitates production of inbred lines with higher kernel lysine and tryptophan content, which could be achieved either through development of entirely new inbred lines or conversion of agronomically elite maize inbred lines through introgression breeding. Initially, conventional breeding was directed toward development of reliable new inbreds with higher kernel lysine content. Owing to higher protein quantity and quality of maize kernel germ/embryo, efforts were made to increase the germ-to-endosperm ratio and selection for multiple aleuron layers. However, the germ-to-endosperm ratio had a negative association with the shelf life of maize. Additionally, many of these efforts were based on recurrent selection. The population-based breeding approaches for enhancement of lysine content through alteration of kernel phenotype were not much successful owing to narrow genetic base and high demand for resources and laboratory facilities (Babu and Prasanna 2014).

The discovery of nutritional importance of *opaque* mutants leads to accelerated breeding efforts for the development of high-lysine-rich maize. High-lysine *opaque* mutants provided avenues to exploit the double *o2* mutants simultaneously in com-

bination with endosperm and amino acid modifiers. The double *o2* mutants in combination with modifiers lead to development of high-lysine maize with vitreous kernel (Vasal 2001). Analysis of endosperm modification of maize kernels in segregating generations was started in 1969 by Lonnquist and Asani (Babu and Prasanna 2014). The initial approaches for development of QPM stocks were based on population improvement strategies, namely, intrapopulation selection for kernel modification *o2* background and recombination of superior vitreous *o2* families. These strategies were employed in large-scale development of QPM donor stocks in temperate, tropical, and sub-tropical germplasm (Vasal 2001). More recently, novel conventional breeding approaches such as "modified backcross-cum-recurrent selection" and "pedigree backcrossing scheme" were employed for rapid and efficient conversion of inbred lines for *o2* and kernel hardiness (Vasal 2001). Presently, pedigree and backcross breeding with QPM × QPM- and QPM × non-QPM-based crosses are used for the development and conversion of QPM inbred lines. Efforts to develop QPM hybrids at CIMMYT were initiated during 1985 owing to advantage of hybrids as compared to OPV-QPM. In addition to higher yield, stable and uniform endosperm modification, and seed purity, QPM hybrids also ensured minimal efforts to monitor the protein quality owing to their genetic uniformity (Vivek et al. 2008). Several QPM heterotic combinations were tested in national and international breeding programs, and some of them were released for regular cultivation. The list of QPM cultivars developed through conventional breeding are collated in Table 7.1.

The promising QPM composites and hybrids developed at CIMMYT were introduced in India, and after testing in All India Coordinated Project on maize, they were released for commercial cultivation. Apart from this, several QPM hybrids were developed in Africa as well as in India from the parental lines supplied by SK Vasal. Efforts were also made to improve the QPM hybrids for drought stress. The screening of QPM genotypes for drought stress identified several germplasms as source for drought tolerance breeding and early maturity (Zaidi et al. 2008; Pfunde and Mutengwa 2016). The inbred CML18 showed early maturity under drought stress (Pfunde and Mutengwa 2016). The heterotic combinations of QPM inbreds, namely, CML3 × CML13 and CML5 × CML9, gave better grain yield (~3 t/ha) under drought stress (Pfunde and Mutengwa 2016). Breeding program for grain yield and tolerance against other stresses is also being carried out by several research groups.

7.4.2 Molecular Marker–Assisted Breeding (MAB)

Although conventional breeding strategies have been used to develop a large number of QPM stocks and convert commercial lines to QPM forms, the procedures are tedious and time consuming. In order to overcome breeding difficulties associated with recessive nature of *o2* and modifiers such as large number of generations during backcross program, rigorous biochemical testing after every generation, selection for multiple endosperm modifiers, and demand for huge resources lead to look for an alternative approach. Advances in genomics science and technology

Table 7.1 List of some of the QPM cultivars developed through conventional breeding

S. No.	Cultivar	Type of cross	Pedigree	Endosperm	Year	Country	Grain
1	AMH760Q (Webi)	Three-way	–	Hard	2010	Ethiopia	White
2	AMH852Q	Three-way	–	Hard	2016	Ethiopia	White
3	BHQP542 (Gabissa)	Three-way	(CML144 × CML159) × CML 176	Hard	2001	Ethiopia	White
4	BHQPY545 (Kello)	Single	CML161 × CML165	Hard	2008	Ethiopia	Yellow
5	BHQPY548	Three-way	–	Hard	2015	Ethiopia	White
6	GH-132-28	Single	P62 × P63	Hard	1997	Ghana	–
7	HB- PROTICTA	Three-way	(CML144 × CML159) × CML176	Hard	–	Guatemala	White
8	HQ INTA- 993	Three-way	(CML144 × CML159) × CML176	Hard	–	Nicaragua	White
9	HQ-31	Three-way	(CML144 × CML159) × CML176	Hard	–	Honduras	White
10	HQ-61	Three-way	(CML144 × CML159) × CML176	Hard	1999	El Salvador	White
11	HQPM-1	Single	HKI193-1 × HKI163	Hard	2005	India	Yellow
12	HQPM-4	Single	HKI-193-2 × HKI 161	Hard	2010	India	Yellow
13	HQPM-5	Single	HKI163 × HKI 161	Hard	2007	India	Yellow
14	HQPM-7	Single	HKI193-1 × HKI161	Hard	2008	India	Yellow
15	ICA	Three-way	(CML144 × CML159) × CML176	Hard	–	Colombia	White
16	KH500Q	Three-way	(CML 144 × CML 59) ×CML181	Hard	–	Kenya	White
17	KH631Q	Three-way	(CML 144 × CML 159) × CML 182	Hard	–	Kenya	White
18	Lishe-H1	Three-way	(CML144 × CML159) × CML176	Hard	–	Tanzania	White
19	Lishe-H2	Top	Obatampa × (CML144 × CML159)	Hard	–	Tanzania	White
20	Melkassa 1Q	OPV	–	–	2013	Ethiopia	Yellow
21	Melkassa 6Q	OPV	–	–	2008	Ethiopia	White
22	MHQ138	Three-way	–	Hard	2012	Ethiopia	White
23	Pratap QPM Hybrid-1	Single	DMRQPM-106 × HKI-193-1	Hard	2013	India	Yellow

(continued)

Table 7.1 (continued)

S. No.	Cultivar	Type of cross	Pedigree	Endosperm	Year	Country	Grain
24	Protina	Composite	(Jowatigua x Antigua car II) o2 x (Doeto × G.C.C.) o2	Soft	1971	India	Yellow
25	Quian 2609	Single	Tai 19/02 × CML171	Hard	–	China	Yellow
26	Rattan	Composite	J1o2	Soft	1971	India	Yellow
27	Shakti	Composite	JLo2, Cuba1Jo2, Antigua 2Do2	Soft	1971	India	Yellow
28	Shakti-1	Composite	Antigua, Ver 181 HEo2, Amarillo crstallino HEo2, Ant Rep Dom, HEo2, temperate HEo2	Hard	1997	India	Yellow
29	Shaktiman-1	Three-way	(CML142 x CML150) x CML186	Hard	2001	India	White
30	Shaktiman-2	Single	CML176 × CML186	Hard	2004	India	White
31	Shaktiman-3	Single	CML161 × CML163	Hard	2006	India	Yellow
32	Shaktiman-4	Single	CML161 × CML169	Hard	2006	India	Yellow
33	Shaktiman-5	Single	CML161 × CML165	Hard	2013	India	Yellow
34	Zhong Dan 206	Single	–	Hard	1988	China	–
35	Zhong Dan 9409	Single	Pool 33 × Temp. QPM	Hard	–	China	Yellow
36	ZS261Q (CZH01021)	Double	(CZL01006 × CML176) × (CZL010 05 × CML181)	Hard	–	Zimbabwe	White

facilitated the adoption of marker-assisted selection (MAS) for rapid and efficient conversion of commercial cultivars into QPM version (Ribaut and Hoisington 1998; Xu and Crouch 2008; Gupta et al. 2009). MAS use molecular markers to select or deselect the target genomic region (*o2*) involved in expression of desired trait without or mere disturbance of the background genomic region. Foreground selection (using gene-based/gene-linked markers) of MAS helps to retain *o2* allele in segregating generations and background selection (using markers polymorphic between the donor and recurrent parents) that aid in recovering individuals with desired genotype at the target locus. Cloning and molecular characterization of *o2* gene identified three *o2*-based SSR markers, namely, phi057, phi112 and umc1066 (Lin et al. 1997; Bantte and Prasanna 2003; Yang et al. 2008). The identified SSRs offered remarkable advantage in foreground selection. "Foreground selection" for the *o2* allele using gene-based SSR markers and "background selection" (using markers polymorphic between the donor and recurrent parents) aid in recovering individuals with desired genotype at the target locus, besides high levels of recovery of recurrent parent genome, within two backcross generations. Babu et al. (2005) and Gupta et al. (2009) developed MAS-based breeding pipeline for rapid conversion of commercial non-QPM inbreds to QPM version within two generation of backcrosses and phenotypic selection for kernel modification and agronomical and biochemical traits in two subsequent selfed generations (Fig. 7.1). However, it was experienced by our team at ICAR-VPKAS, Almora that the number of markers for background selection needs to be large (preferably >100) for a viable MAS-based breeding approach. This strategy allows selection and fixing of large segregating generation for the *o2* in segregating generations with simultaneous reduction of linkage drag by using flanking markers for recipient allele types. This strategy can thus be implemented in a cost- and time-effective manner as compared to phenotypic selection alone (Dreher et al. 2003; Babu and Prasanna 2014). The flow chart in Fig. 7.1 shows the schematic diagram for the same.

Availability of simple, straightforward MAS strategy and low cost of genotyping enabled the plant breeders to adopt MAS as a method of choice for rapid development of QPM version for the development of commercial cultivars. Additionally, it also facilitated the diversification of genetic base of QPM cultivars to suit the need of targeted agroecologies. There are very good number of successful examples of MAS for the development of QPM hybrids available in Indian maize breeding programs (Table 7.2) (Babu et al. 2005; Gupta et al. 2009, 2013; Prasanna et al. 2010). Using the method, many normal maize inbreds and hybrids have been converted into QPM versions and were released for commercial cultivation (Babu et al. 2005; Gupta et al. 2009). Five successful examples of developing QPM hybrids that were converted through MAS are presented below.

Vivek QPM 9 Gupta et al. (2009) converted successfully a promising maize hybrid, namely, Vivek Maize Hybrid 9, to QPM version using MAS. Vivek QPM 9, the resulting QPM hybrid, matures in 85–90 days and yields up to 6.0 t/ha. The performance of Vivek QPM 9 (5.8 t ha^{-1} in zone I and 5.4 t ha^{-1} in zone IV) was at par with Vivek Maize Hybrid 9 (5.9 t ha^{-1} and 5.4 t ha^{-1} in zone IV) in both the zones over years. It

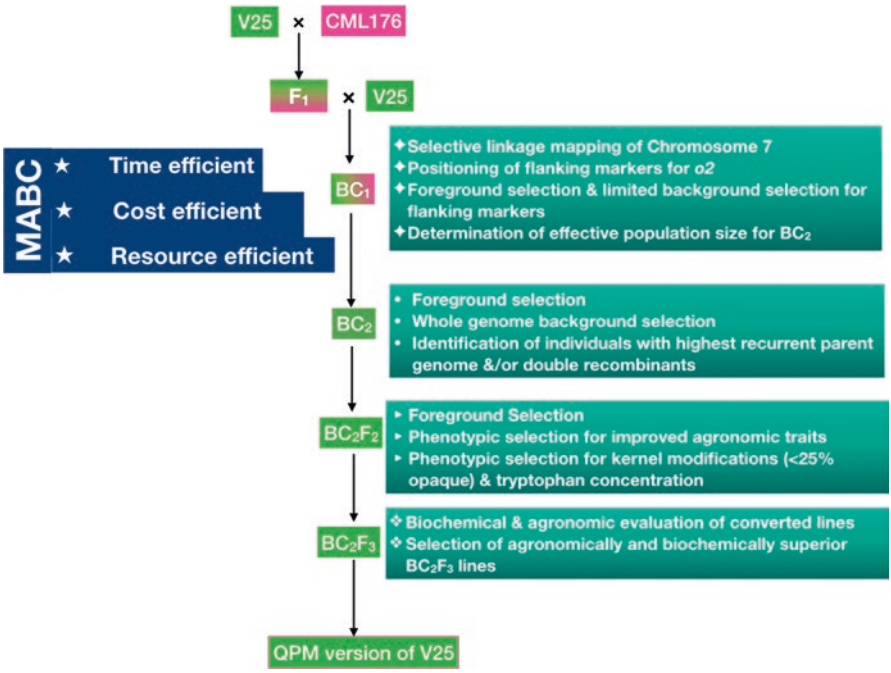

Fig. 7.1 Schematic diagram for converting a normal maize inbred (V25) to QPM version through MAS. (Modified from Babu et al. 2005)

also showed 41% higher tryptophan in the endosperm as compared to Vivek Maize Hybrid 9. It possesses equal level of resistance to *turcicum* blight – the most important disease of maize crop in the hills. Based on the performance for 2 years in zone 1 and zone IV, Vivek QPM 9 was released in 2008 for commercial cultivation in zone I and zone IV in India and for the organic conditions in the hills of Uttarakhand (Fig. 7.2).

Vivek QPM 21 Vivek QPM 21 (QPM version of Vivek Maize Hybrid 21) shows >70% enhancement in tryptophan over the original hybrid, Vivek Maize Hybrid 21. The tryptophan content of Vivek QPM 21 is 0.85, whereas it is 0.49 for Vivek Maize Hybrid 21. Vivek QPM 21 was also tested in the All India Coordinated Trial of *Kharif* 2007, 2008, and 2009, in which it performed equally well in respect to grain yield and other agronomic traits over non-QPM national check, Vivek Maize Hybrid 17 and Vivek Maize Hybrid 21. Vivek Maize Hybrid 21 was released for commercial cultivation in zone I, II, and IV in 2006. The parents of this hybrid were converted into the QPM version using DNA markers, and this hybrid was reconstituted by crossing VQL 1 and VQL 17. This QPM hybrid shows more than 70% enhancement in tryptophan over the original hybrid. In the state trials of Uttarakhand under organic condition, this hybrid gave more than 2.4% higher grain yield and was released for the state of Uttarakhand, India, in the year 2012 for commercial cultivation by the State Varietal Release Committee, Uttarakhand, for the hill conditions (Fig. 7.3) (Agrawal et al. 2015).

Table 7.2 MAS-derived single-cross QPM hybrids released for commercial cultivation in India

S. No.	Cultivar	Original Hybrid	Endosperm and Grain Color	Institute	Year
1.	Vivek QPM 9	Vivek Hybrid 9	Hard and Yellow	VPKAS, Almora	2008
2.	Vivek QPM 21	Vivek Hybrid 21	Hard and Yellow	VPKAS, Almora	2010
3.	Pusa HM4 Improved	HM4	Hard and Yellow	IARI, New Delhi	2017
4.	Pusa HM8 Improved	HM8	Hard and Yellow	IARI, New Delhi	2017
5.	Pusa HM9 Improved	HM9	Hard and Yellow	IARI, New Delhi	2017

Fig. 7.2 A crop of Vivek QPM 9

Pusa HM4 Improved This QPM hybrid contains 0.91% tryptophan and 3.62% lysine, which are significantly higher than the popular normal non-QPM hybrids. It matures in 87 days with an average yield of 6.4 t ha^{-1}. This hybrid was developed at the ICAR-Indian Agricultural Research Institute, New Delhi, and released for commercial cultivation in the year 2017 for summer (*Kharif*) season in the states of Punjab, Haryana, Delhi, plains of Uttarakhand, and western region of Uttar Pradesh.

Pusa HM8 Improved This is a newly developed QPM hybrid rich in tryptophan (1.06%) and lysine (4.18%) which is more than double of the popular non-QPM hybrid. It yields 6.3 t/ha in 95 days. The hybrid was developed at the ICAR-Indian Agricultural Research Institute, New Delhi, tested for 2 years under All India

Fig. 7.3 Cobs of Vivek QPM 21

Coordinated Research Project on maize and released for commercial cultivation in summer (*Kharif*) season in the states of Maharashtra, Andhra Pradesh, Telangana, and Tamil Nadu of Indian Union in the year 2017.

India is one of the first few countries to focus on *o*2 maize and released three *o*2 composites, namely, Shakti, Rattan, and Protina in 1970 (Dhillon and Prasanna 2001) and one modified superior *o*2 composite "Shakti 1" in 1997. Later on, India released several QPM hybrids *viz*., Shaktiman-1 (a three-way cross hybrid), Shaktiman-2, Shaktiman-3, Shaktiman-4, HQPM-1, HQPM-5, and HQPM-7 (single cross hybrids). Vivek QPM 9 was the first MAS-derived QPM hybrid released in the year 2008. It was followed by the release of another four QPM hybrids (Agrawal and Gupta 2010). Three popular hybrids were recently converted into QPM and were released for commercial cultivation in the year 2017. All the QPM composites and hybrids, with major traits released in India, are presented in Table 7.2.

7.5 QPM in Human and Animal Nutrition

The WHO (2007) suggested the daily requirement of 0.66 and 0.73 g protein/(kg BW•d) requirement for adults and infants, respectively. Correspondingly, the recommendations were also given for essential amino acids, namely, lysine (adult, 30 mg/(kg•d); infants, 35–45 mg/(kg•d)) and tryptophan (adult, 4 mg/(kg•d); infants, 4.8–6 mg/(kg•d)) (Institute of Medicine 2006). By virtue of two- to three-fold higher lysine and tryptophan content than non-QPM, QPM poses higher

biological value, balanced nitrogen index, and leucine/isoleucine ratio for better niacin synthesis (Ma and Nelson 1975; Vivek et al. 2008). The biological value depicts the amount of nitrogen retained in the body. The QPM poses exceptional biological value of 80% as compared to 40–57% in normal maize (Bressani 1992). The results were further confirmed by Abiose et al. (2015), where QPM-based diets showed better biological value (>60%) and digestibility (>60%) than the normal maize products. Owing to significant nutritional benefits, QPM could serve as an excellent source to overcome protein-energy malnutrition in humans and also better feed for livestocks. Additionally, replacement of common maize with QPM provides a more balanced protein source without scarifying energy, grain yield, and micronutrients and changing native food supply systems and economic benefits to farmers (Nuss and Tanumihardjo 2011).

7.5.1 QPM as Food for Human Being

Owing to the higher biological value of QPM, it was recommended as food to help in reducing protein deficiency. Accordingly, the QPM brought back the children suffering from kwashiorkor, a severe protein deficiency syndrome to normalcy in Columbia (Bressani 1990). QPM can also be used as an ingredient in the preparation of composite flours to supplement wheat flour for chapatti, bread, and biscuits preparation. In many countries such as in many countries such as Brazil, Zambia, Zimbabwe, and Ghana ten percent maize flour has been used in composite flours. According to a recent study in rural Ethiopia on the nutritional benefits of QPM and its acceptance as a food, when QPM was supplemented in food as their main starchy staple, the heights and weights of preschool children increased more than 20% faster than those of children who ate conventional maize. Maize is becoming a major staple food in Ethiopia as the price of tef and mdash, the traditional indigenous food, is rising beyond the means of resource-poor consumers, and their yields generally fall short of household needs. Rural communities rely more and more on maize for both calories and protein, especially where people lack access to other protein sources (Anon 2008).

Six-month feeding study of QPM and non-QPM conducted on children at Rajendra Agricultural University, Pusa of Bihar state in India, revealed increased body weight and arm circumference of children fed with QPM which was remarkably higher as compared to children fed with non-QPM (Mamatha et al. 2017). Similarly, in Uttar Pradesh another state of India, a QPM feeding experiment of 180 days on children (1–3 years age) revealed higher weight, height, head circumference, chest circumference, and arm circumference as compared to non-QPM, milk fed, and control diets (Chopra et al. 2011). On the other hand, studies conducted on children in Ethiopia revealed a positive effect on weight but no improvement for height (Akalu et al. 2010). A meta-analysis of community-based studies on consumption of QPM in Africa and India revealed that consumption of QPM instead of non-QPM leads to increase in the growth rate of 12% and 9% for weight and height, respectively, in infants and young children with mild to moderate undernutrition

(Gunaratna et al. 2010). Sixteen weeks of supplementation of QPM-based composite diets resulted in significant improvement in height, weight, weight-for-age, height-for-age, and weight-for-height z-scores and hemoglobin levels among the children receiving such diet with QPM (Maseta 2016). The consumption of QPM not only make the target population nutritionally sufficient but economically viable too.

A general conclusion about QPM is that it is superior to normal maize on its amino acid balance and nutrient composition and could improve the performance of various animals. It is more economical to use diets incorporating QPM since there will be a reduction in the use of fish meal and synthetic lysine additives. While studying the nutritional factor of QPM, together with soybean flour, brown sugar, banana meal, and oat meal, for Fisher rats, observed that QPM showed a good potential for utilization in nutritional supplements, especially when associated with soybean flour.

7.5.2 QPM as Feed in Poultry and Livestock

Maize is well accepted as the king of the feed ingredients. It is a primary source of energy supplement in animal diets and can contribute up to 30, 60, and 90% of the diet's protein, energy, and starch, respectively (Dado 1999). About 70–80% of maize production is used as a feed ingredient in the world. While comparing the feed value of normal maize (NM) and quality protein maize (QPM), Zhai (2002) reported that there was no significant difference on gross energy (GE), apparent metabolizable energy (poultry), and apparent digestible energy (pig) content between QPM and NM. Although NM had a higher GE content than QPM, its contents of AME (apparent metabolizable energy) for poultry and ADE (apparent digestible energy) for pig were lower than those of QPM. This indicates that the energy availability of QPM is little higher than that of NM.

Many investigations show superiority of QPM as source of feed for dairy and piggery. Although, chemical composition of fodder from QPM was similar to that of the normal maize, fodder of QPM varieties (HQPM 5, HQPM 7, and HQPM 9) showed better in vitro digestibility values (IVDMD% and IVOMD%) than other normal maize varieties (Vaswani et al. 2015). Studies conducted at International Livestock Research Institute, Debre Zeit Research Station, in Ethiopia, showed that QPM silage-based diet was superior in dry and organic matter degradability and had higher ammonia and volatile fatty acids concentration than normal maize stover-based diet (Tamir et al. 2012).

7.5.2.1 QPM as Feed for Layers

Zhai (2002) found that replacement of NM by QPM significantly enhanced the egg production. The QPM-based diets also increased the feed intake of the birds remarkably. The utilization of QPM in laying hen also enhanced the yolk color pigmentation. However, no significant effect was found on the weight of the eggs. Similarly, Osei

et al. (1999) reported that when QPM was added to pullet diets, the protein level can be reduced to 14% without any adverse effects on their performance, whereas, the addition of QPM to layer diets has significant effects on the age at first egg production, 50% hen day and housed production. This indicates that the QPM when used in feed result in considerable financial benefits without sacrificing performance.

7.5.2.2 Nutritional Superiority of QPM in Broilers

Osei et al. (1998) conducted two experiments, each lasting 6 weeks, to evaluate QPM as a feed gradient for broiler chickens. In Experiment 1, either normal hybrid maize or QPM was used as the sole source of protein and amino acids, and the diets were balanced for vitamins and minerals by the addition of a vitamin-mineral premix. These diets were compared with a balanced 21% crude protein starter-finisher broiler diet. In Experiment 2, broiler chicks were fed with combined starter-finisher diets of varying protein contents in which QPM replaced normal maize. The aim was to assess the potential for reducing dietary fish meal incorporation as QPM replaced normal maize. In both the experiments, feed and water were supplied ad libitum. The parameters measured included feed intake, growth rate, feed conversion efficiency, and carcass characteristics. The economics of broiler production was determined in Experiment 2. In Experiment 1, birds receiving QPM as the sole source of amino acids performed significantly better than their counterparts fed on normal maize. QPM-fed birds weighed an average 708.0 g each at the end of the trials compared with 532.0 g for those on normal maize. The corresponding feed efficiencies were 4.28 and 6.55, respectively. Compared with birds on the balanced diet, however, QPM was inadequate in supporting broiler growth. In Experiment 2, the use of QPM allowed the fish meal to be reduced from 19.5% to 13.5% without adversely affecting performance. They also found it cheaper to produce broilers using QPM than using normal maize. Similarly, Bai (2002) reported that the dietary replacement of normal maize by QPM significantly increased weight gain of days 21–42, 42–49, and 1–49 in broilers. They concluded that using QPM to replace normal maize in the broilers diet may have economic benefit due to improved weight gain, FCR, and decreasing of dietary lysine supplementation. In another study, dietary replacement of non-QPM with 50% QPM resulted significant improvement in body weight gain, feed conversion ratio, immune response, relative bursa weight, and breast muscle yield and lowered abdominal fat content of broilers (Panda et al. 2010). The feeding of QPM over non-QPM commercial hybrid *Nithyasri* in broiler chickens resulted improved breast meat yield and serum biochemical profiles and reduced the abdominal fat content and serum cholesterol (Panda et al. 2011).

7.5.2.3 Nutritional and Biological Superiority of QPM in Pigs

Nutritional studies of QPM has also been made in pigs. In a study by Zhai (2002), it was observed that the QPM had higher apparent and true ideal amino acid digestibility of most amino acids than those of NM in pigs. QPM had not only a higher

content of lysine but also higher digestibility of lysine for pigs. The reason for this increased digestibility is possibly due to increase of albumins/globulins ratio. However still methionine is one of the limiting amino acids in maize (both QPM and NM) when used as animal feed. Gao (2002) made a similar study in pigs and reported that in grower phase (20–50 kg), replacement of NM by equal ratio of QPM in pig diets significantly improved the average daily gain (ADG). This indicates that QPM as a feed is better than NM. This is due to higher lysine content and better digestibility of critical essential amino acids. However, replacement of NM by QPM had no significant effect on carcass characteristic of pigs. A QPM feeding trial on weaner pigs showed higher feed conversion efficiency of weaner pigs fed with QPM diet and also cheaper cost of production owing to QPM-based protein source (Mpofu et al. 2012). In addition to the direct impact on metabolism of animals, QPM can serve as an excellent substitution for high-value ingredients in feed (soybean meal) diets without adverse effect on animal performance, which helps in reducing cost of animal rearing (Prandini et al. 2011). Similarly, studies on the impact of QPM diet on growth and performance of weaner rabbits revealed that substitution of QPM could reduce the cost of weaner rabbits raising without affecting the growth and metabolism (Omage et al. 2009).

7.5.2.4 Economic Impact of QPM as Feed

Miguel and Pereira from CIMMYT, Mexico, evaluated pig and poultry feed cost and composition effects from including QPM as an alternative energy and protein source. Cost savings could be approximately 3.4% (about $5/ton) for pig feed, with QPM constituting about 80% of the ration and replacing all regular maize and synthetic lysine and 40% of soybean meal. Savings are slightly lower for poultry feed. However, if a 5% price premium for QPM over regular maize is assumed, most of the savings are lost, indicating that QPM should compete at the same price to be economically attractive as a commercial feedstuff (www.cimmyt.org).

7.6 Challenges in Large–Scale Adoption and Area Expansion of QPM

7.6.1 Constraints in Area Expansion Under QPM

QPM is a cost-effective way to improve the diets of protein-malnourished populations, where maize is one of the staple intakes. Several QPM cultivars have been developed in various national and international maize breeding programs and they are being disseminated and promoted. Some of the countries witnessed impressive adaption of QPM from farming community and better consumer acceptance. For instance, in Africa, Ghana is leading the QPM adoption and production with 70,000 ha of QPM area with the vast majority of *Obatanpa* variety (Krivanek et al.

2007). By 2009, more than ~40 countries had released QPM varieties for general cultivation (Sofi et al. 2009). China leads the Asian maize-growing countries, and interest in QPM has grown tremendously with active support of government. More than 100,000 ha are currently planted with QPM hybrids. It is expected that more than 30% of the total maize-growing area in China will be covered by QPM hybrids by 2020 (De-quan and Shihuang 1994). Mexico accounting around 2.5 million hectares of QPM and countries in Africa such as Mali, Burkina Faso, Benin, Ethiopia, Uganda, Mozambique, and South Africa are growing QPM in 5–20 thousand hectares. In developing countries, the QPM varieties have been contributing more than $1 billion annually (Vasal 2002; Nedi et al. 2016). However, despite of numerous QPM varieties released and diverse reports showing the importance of producing QPM on nutritional well-being of humans and farm animals, the adoption has been limited worldwide. Presently, QPM varieties are grown on roughly nine million acres worldwide, which encompass only <1% of the total area under maize production (Rugema 2014). The challenges facing dissemination of the QPM hybrids in the farmer's field includes seed availability, segregated procurement and marketing, procurement-to-consumption chain, policy support for premium prices (to QPM), and policy support to take QPM to consumption chain.

7.6.2 Awareness on Health Benefits of QPM Among Farmers and Consumers

In spite of the fact that the QPM technology is available to the farming community and consumers, majority of the consumers and farmers in developing and underdeveloped nations are not aware of the nutritional benefits of QPM. Intensive awareness campaigns needs to be initiated for making QPM popular among the farmers as well as consumers. Studies conducted in Africa revealed familiarity of several group of farmers to QPM even if they had minimal awareness about its nutritional benefits (De Groote et al. 2016). Approximately 94% of the respondents agreed to consume nutritionally superior yellow maize over white on knowing the health benefits of QPM (Stevens and Winter-Nelson 2008). In addition to spreading of awareness, health benefits of QPM should be spread for the QPM products to increase the consumer preference over conventional maize products.

7.6.3 Incentives and Policy Support to the QPM Stakeholders

Adequate policy supports and incentives from the respective governments are necessary for the success of programs on QPM. Presently, there are no incentives available for the farmers growing QPM and food processing industries using QPM. Therefore, the promotion of QPM requires incentives for QPM farmers and

food processing industries in some or other form. Subsidy for the cost of the QPM seeds and premium price for the QPM grains are viable propositions. The delivery of QPM to target population could be facilitated through food and feed processing industries. Midday meal and "public distribution system" are the other means to reach the QPM products to consumers. Processing and value addition to QPM products need to be encouraged.

7.6.4 Successful Public–Private Partnership

Effective public-private partnership can help in bringing magical adoption of QPM. It should include partnership in development of cultivars, commercialization, processing, value addition, and value chain development. The help of local non-governments, non-governmental organizations, and village self-help groups will be highly useful in this endeavour. In view of the successful spread and adoption of maize hybrids in India during the last two decades during which private sector played major role. It is imperative to rope in private sector for development of maize hybrids whose productivity is at par with that of the normal single cross hybrids. Hand-in-hand, seed production and dissemination of these QPM hybrids should also involve the private sector. In addition, utilization of QPM in preparation of various edible products like breakfast cereals, nachos etc. by the industry, can be accelerated by bringing them on board with the producers and researchers who can, in turn meet the requirement of various parameters of QPM grains.

7.6.5 Adequate and Organized Food Processing Industry

The well-informed consumers were found willing to purchase QPM-derived quality food products with premium price. In Kenya, maize consumers were willing to pay a premium price for biofortified maize (De Groote et al. 2011). Similarly, consumers were observed to pay 20–70% premium price for the biofortified foods developed through genetic engineering (De Steur et al. 2015). QPM-derived foods are expected to have a good demand if consumers are made aware of product from QPM. However, in most of the developing nations, processing facility and related marketing of QPM products are not well organized.

7.6.6 Strong Extension Programs and Creation of "Nutri-Village" Clusters

Strong extension program for the expansion of area under QPM and its acceptability among the consumers is required. Adaptation of strong extension programs could lead to expansion of QPM area up to 70% in Uganda and 30% in Tanzania (De

Groote et al. 2016). Involvement of local peoples, volunteers, and folk artist along with regular extension workers to create awareness about QPM among villagers using mass communications, community drama, road shows, and "field days" could increase its acceptability. Another viable approach for the popularization of QPM is the creation of "Nutri-village" clusters to ensure quality QPM seed production and QPM grains for the industries. The "Nutri-village" should be designed for production of QPM only. They may also be encouraged to go in the mode of "contract farming" for reasonable income of QPM farmers and continuous supply of QPM products for industries. It may be in the line of "Orange day" of Mozambique and Uganda for promotion of orange sweet potato.

An effort was jointly made by VPKAS, Almora, and Hindustan Insecticide Limited (company) by producing around 2000 quintals of QPM seeds involving 168 farm families in the Krishna Nagar, Nadia district of West Bengal, India. This is one of the successful examples for the large-scale production of public sector QPM hybrid, Vivek QPM 9, in seed village hub. The produce was used as seeds in the Northeastern states and West Bengal, in India.

7.6.7 Linking All the Stakeholders on Common "QPM Platform"

QPM has turned out to be an important discovery that has benefitted many African countries in reducing protein malnutrition significantly; however, technology has not been utilized fully in Asis in general and south Asia in particular where protein malnutrition is a major challenge. In order to harness the potential of QPM in Asis, there is a need to bring all the stakeholders like researchers, farmers, industries, seed companies, public organizations, NGOs, and SHGs for successful production, processing, value addition, and marketing of QPM. Support among the stakeholders will help in complementing and supplementing each other for the success of QPM in Asia.

7.7 Way Forward and Conclusions

QPM is one of the practical solutions to fight protein-energy malnutrition and has successfully been deployed in several African countries where maize is a staple food. It has been able to create an impact in reducing protein malnutrition in large population when the acceptability and support to farmers and consumers were available. QPM can, thus, be presented and supported as a better alternative to combat the malnutrition in the regions where maize constitutes a large part of the diet. Many developing countries in Africa and South America have maize as the staple food. Besides, QPM could also be popularized as a source of food among the Asian countries where maize is majorly preferred as animal and poultry feed. In these areas, there are pockets in which maize is consumed as food. Thus, replacing normal

maize with QPM is likely to have salutary impact as seen in Africa. Once a demand for QPM is created, it will help in diversifying cultivated lands from rice to maize especially in upland areas where rice productivity continues to hover around 1.00 t ha^{-1} even today. Furthermore, QPM enriched with Fe, Zn, and provitamin A can be used to overcome hidden hunger along with protein malnutrition (Gupta et al. 2015a, b; Mallikarjuna et al. 2014, 2015; Mallikarjuna 2015). Likewise, in combination with low phytate content, QPM will help in reducing Fe and Zn deficiency. Such a maize cultivar can be called as "super QPM" satisfying protein and micronutrient malnutrition in holistic manner. Recent advances like genome editing (CRISPR/Cas), genomic selection, and other molecular breeding will help in developing "super QPM."

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