Global Science Research Journals

ISSN: 2408-5502 Vol. 4 (8), pp. 295-304, August, 2016

http://www.globalscienceresearchjournals.org/

Global Journal of Animal Breeding and Genetics

Review

A study on Quality protein maize (QPM) and Genetic management for the nutritional protection of maize

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Accepted 29 July, 2016

Cereals are the only source of nutrition for one-third of the world's population especially in developing and underdeveloped nations of Sub-Saharan Africa and South-east Asia. The three major cereals, rice, wheat and maize constitute about 85% of total global cereals production amounting to about 200 million tonnes of protein harvest annually at an average of 10% protein content, out of which a sizeable proportion goes into human consumption (Shewry, 2007). A major concern in case of developing nations is that in most cases, a single cereals crop is the major food staple and as such the nutritional profile of cereal crops assumes great significance. Grain protein content of cereals has a very narrow range with rice (5.8-7.7%), maize (9-11%), barley (8- 15%) and wheat (7-22%) as reported by various workers. In many developing countries of Latin America, Africa and Asia, maize is the major staple food and often the only source of protein. At global level, maize accounts for 15% of proteins and 20% of calories in world food diet. But unfortunately, the nutritional profile of maize is poor as it is deficient in essential amino acids such as lysine, tryptophan and methionine due to a relatively higher proportion of prolamines in maize storage proteins which are essentially devoid of lysine and tryptophan. The reason concerning this is that lysine, tryptophan and threonine are the limiting amino acids in human beings and non -ruminants. Maize is also an important component of livestock feed especially in developed nations where 78% of total maize production goes into livestock feed. Therefore, breeding strategies aimed at improving the protein profile of maize will go a long way in reducing prevalence and persistence of malnutrition in developing world.

Key words: *Mucronate,* protein, maize, zein.

INTRODUCTION

Storage proteins in maize

The maize grain largely consists of endosperm that is rich in starch (71%). Both the embryo and endosperm contain proteins but the germ proteins are superior in quality as well as quantity. 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. The maize endosperm consists of two district regions having different physical properties. The aleurone layer is the outer most layer rich in hydrolytic enzymes secreted by specialized cells. Within the aleurone layer is the starch rich endosperm having vitreous and starchy regions. The zein proteins found in vitreous region form insoluble accretions called protein bodies in the lumen of rough endoplasmic reticulum and towards maturation are densely packed between starch grains (Gibbon and Larkins, 2005). These zeins consist of albumins, globulins, glutelins and prolamins and constitute about 50-60% of maize proteins. The prolamins are rich in proline and amide nitrogen derived from glutamine. All prolamins are alcohol soluble (Shewry and Halford, 2002). The prolamins of maize grain are called zeins and consist of one major class (α-zeins) and three minor classes (β , and δ). The zein fraction α is rich in cystein while β-and -fractions are rich in methionine. These four types

α, β and δ constitute about 50-70% of maize endosperm and are essentially rich in glutamine, leucine and proline and poor in lysine and tryptophan. Other proteins such as globulins (3%), glutelins (34%) and albumins (3%) are collectively called non-zeins (Figure 1). The zein fraction in normal maize normally contains higher proportion of leucine (18.7%), phenyla-lanine (5.2%) isoleucine (3.8%), valine (3.6%) and tyrosine (3.5%), but smaller amounts of other essential

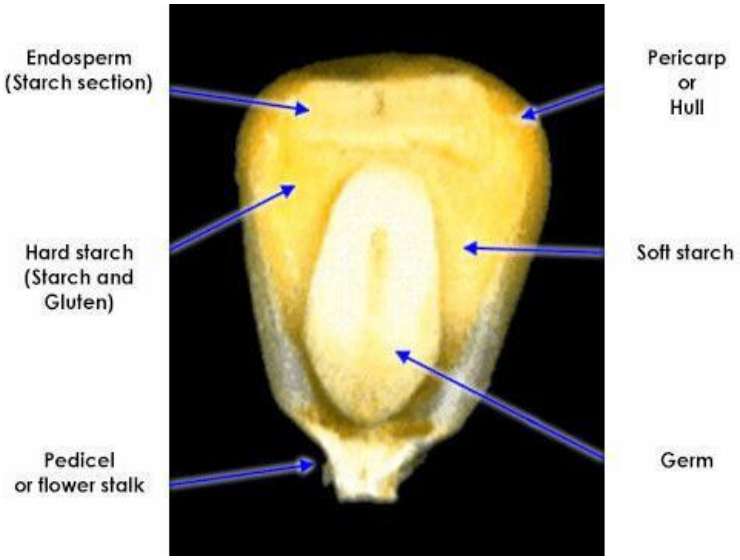

Figure 1. Structure of maize kernel (Source: www.fao.org).

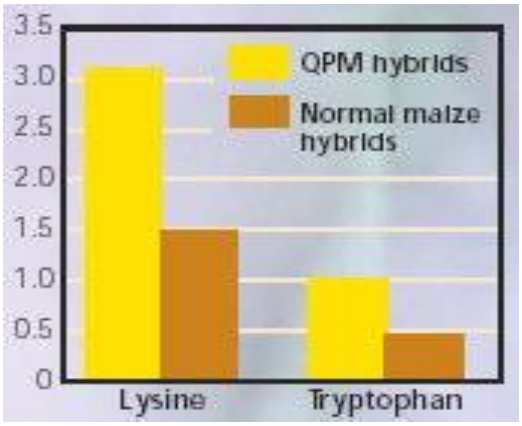

Figure 2. Comparative composition of normal and *o2* maize for lysine and tryptophan Genetic manipulation of protein quality.

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. The non-zein protein f raction is balanced and rich in lysine and tryptophan (Vasal, 2000). The zeins are synthe-sised on rough endoplasmic reticulum membrane and accumulate as protein bodies in its lumen (Figure 2).

With an objective to screen out maize lines containing improved amino acid balance in endosperm to enhance its biological value, efforts were directed in early 1900 to identify such genotypes. Two major problems were faced: one that no specific genes were identified that governed the amino acid profile of maize proteins, and could be used in breeding programmes. Secondly the lack of a simple genetic system precluded the use of backcross programmes to improve upon protein quality in maize. However, in 1920, a naturally occurring maize mutant was identified in Connecticut maize fields in USA that had soft and opaque grains and was named as opaque 2 (*o-2*) (Singleton, 1939). In 1960, Nelson and Mertz worked with the mutant lines at Connecticut Experiment station to identify maize lines with improved protein profile (Krivanek et al., 2007). In 1961, Researchers (researchers) at Purdue University observed that mutant lines that were homozygous for *o2* allele had significantly higher lysine (almost double) in endosperm compared to normal maize. These discoveries aroused great enthusiasm and hope among researchers towards genetic manipulation of protein quality in maize and resulted in discovery of various other mutant types that had altered amino acid com-position. These include the *floury-2 (fl-2), Mucronate (Mc*) and *Defective endosperm B30 (DE B30).* The opaque mutants are recessive *(o1, o2, o5, o9 -11, o13, o17),* the floury mutation is semidormant *(fl-1, fl-2 and fl- 3)* where as *Mucronate* and defective en-dosperm are dominant mutations. Figure 3 shows the position of zein genes and the mutations on maize gene map. The opaque mutations affect the regulatory network whereas floury; *Mucronate* and defective endosperm affects the storage proteins (Gibbon and Larkin, 2005). The improved protein quality of such mutants was apparently due to increase in proportion on non-zein fraction that is rich in lysine and tryptophan and repression of zein synthesis. Each of the zein polypeptide is a product of differential structural gene (Zp). These zp genes are simply inherited and are members of a large group of genes (upto 150). In terms of zein repression o7 >o2 > fl-27> De B30. Epistatic interactions have also been reported among various regulatory mutants (Prassana and Sarkar, 1991). Thus o2 and o7 are epistatic over fl-2, whereas o2 and Mc have synergistic effect. The reduction in levels of zeins by various mutant allele is accomplished either by reduction in levels of various zein sub-units, rate of

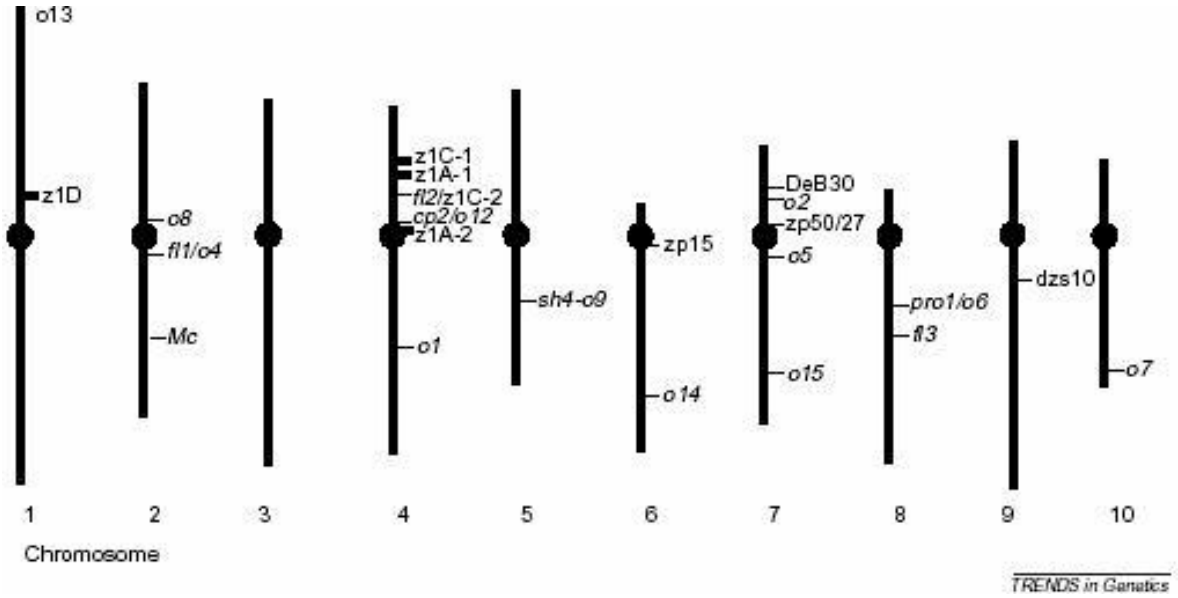

Figure 3. Chromosomal locations of various zein genes (source: Gibbon and Larkin, 2005).

accumulation of zeins, increase in methionine content and effect on timing and pattern of storage protein accumulation. The only mutant gene conditioning protein quality in maize is O2 which encodes a defective basic-domain-leucine-zipper transcription fact or and has been mapped to short arm of chromosome 7. In its dominant form o2 regulates the expression of 22 KDa α-zeins (Damerval and Eevienne, 1993) and other genes including lysine ketoglutarate reductase the mutant allele 02 typically causes a significant increase in non-zein fraction associated with decrease in lysine-poor α-zein proteins. Moreover, the defective LKR enzyme causes increased levels of free lysine. In normal maize, the free amino acids account for a low proportion of total amino acids in normal grains. The amino acids lysine threonine and metheonine are synthesized from asparatic acid (Shewry, 2007). There are complex regulatory networks that maintain low levels of free amino acids in grains by feedback inhibition. The increased levels (level) of lysine in *o2* mutants is due to higher levels of an elongation factor of protein sysnthesis (eEF1A). This factor though itself being rich in lysine (10%) but accounts for only 2% of lysine in the endosperm. Therefore, it is evident that the higher expression of eEF1A is accompanied by a number of transcription factors. QTL mapping studies have revealed linkage between eEF1A and genes encoding zein storage proteins (Valenzualla et al., 2004). The floury-2 mutants *(fl-2)* encode a 22KD α -zein gene with a defective signal peptide that causes mutant polypeptide to accumulate in the membrane of endoplasmic reticulum (Copleman et al., 1995). Similarly, in case of defective endosperm mutants (De B30), a 19 KDa α-zein is produced that also has of a defective signal peptide (Gilkin et al., 1997). However, in case of *Mucronate (Mc)* mutants, there is a frame shift mutation in 16 KDa -zein. All these mutants together cause disruption in development of vitreous texture of maize kernel and result in opaque phenotype. The recent studies using RNA interference based silencing of 22 and 19 KDa RNAi lines more profoundly caused opaque phenotype as compared to 19 KDa component. This is probably due to greater interaction of 22 KDa components with β and -zeins (-zeins) resulting in disruption in protein body formation which causes the opaque phenotype (Segal et al., 2003; Huang et al., 2004).

Problems associated with high lysine mutants

Even though high lysine mutants aroused tremendous interest and enthusiasm for their possible use in developing maize with superior protein quality, but rapidly the negative plieotropic (pleiotropic) effects of such mutations began to be recognised. These undesirable features were limiting factors to its widespread use and adoption (Lauderdale, 2002). Even though the endo-sperm protein mutants such as *o2* and *fl-2* favourably change the amino acid profile of maize grain, they also cause certain undesirable consequences, as is expec-ted of most mutants. The undesirable characteristics include reduced yield than normal maize, low grain consistence and a farinaceous endosperm that retains water (Toro et al., 2003). These features result in a soft, chalky endosperm that dried slowly making it prone to damage, a thick pericarp, more susceptibility to disea-ses and pests, higher storage losses and also affects harvest ability. Since the kernel weight is reduced due to less density per unit volume as starch is loosely packed with lot of air spaces, there is corresponding decline in the yield (Singh and Venkatesh, 2006) which can be almost to the tune of 10 percent or above. Especially in developing countries, where farmers are

accustomed to hard flint and dent grains, the kernel appearance of such mutants made it less ideal for large scale use and adoption in target areas. The mutations that alter grain protein synthesis cause changes in texture of grains. The early opaque-2 (o2) mutants had reduced levels of α-zeins resulting in small unexpanded protein bodies (Geetha et al., 1991), whereas, o15 that reduces -zeins leads to smaller number of protein bodies. Other mutations such as floury-2 *(fl-2), Mucronate (Mc)* and defective endosperm (De B30) result in irregularly shaped protein bodies.

In order to overcome these apparent limitations for large scale use of such mutants, efforts were directed towards identification of alternative mutants that did not carry such disadvantages. This resulted in identification of additional mutants of opaque and floury series, even though none of them eventually could get to farmers fields. This dampened the spirit with which high lysine maize research was pursued as the complexity of the coordinate gene action governing endosperm protein profile became more and more evident. Another major set back to opaque-2 mutant research came in 1973 when WHO and UN revised the energy and protein recommendations. The energy was given more priority over protein for defining them as major limiting factors in malnutrition (Lauderdale, 2002). As a result, the interest of researchers got distinctly polarised towards high energy rather than high protein, therefore, a renewed focus towards higher yields to meet energy demands.

From opaque-2 to QPM: Endosperm modifiers

The undesirable traits associated with opaque-2 mutation coupled with the de-emphasis of protein requirements, the interest in *o-2* was virtually off the ground but researchers at CIMMYT and elsewhere continued their efforts to develop maize that had high lysine and tryptophan and could favourably compete with normal maize for yield and grain characteristics. Under the dynamic leadership of Dr. S.K. Vasal, Researchers at CIMMYT, and University of Natal, RSA, various endosperm modifier genes were identified that could favourably alter the grain characteristics, thereby overcoming an important obstacle in popularisation of high lysine *o-2* maize. These modifier genes do not have any effect of their own as such but interact to improve the kernel hardiness and appearance and increase kernel weight and density. A large number of reports followed wherein varying degrees of endosperm modifications were observed (Paez et al., 1969; Anapurna et al., 1971). This ends up with the idea that such endosperm modifier genes could be used along with *o-2* gene either singly or in combination with other mutants such as sugary- 2 (Su-2) in order to have acceptable characteristics in the final product. Such combinations resulted in maize lines that possessed high lysine, vitreous grains and better protein digestibility even though yield was affected.

The mechanism by which the endosperm modifiers change the grain structure from chalky to vitreous in modified opaque-2 (mo2) is not clearly understood, neither have the genes been characterised in terms of their number, chromosomal locations, nor expression levels in different genetic backgrounds, even though Lopes et al. (1995) linked two chromosomal regions in chromosome 7 with endosperm modification. The o2 mutants that have reduced levels of 22 KDa α -zeins, mo-2 mutants have 2 -3 times higher levels of 27 KDa -zeins (Geetha et al., 1991). The higher level of -zeins is thought to initiate the formation of protein bodies, due to disulphide bond mediated cross linking of 27 KDa -zeins with other cysteine-rich proteins. The observation was that Moro et al. (1995) the modifier genes increased the levels of 27 KDa -zeins in opaque-*2* backgrounds only, indicating thereby that such genes do not, per se, improve the protein quality of maize (Moro et al. 1995). Moreover, they found that higher level of -zeins increased the hardness to a particular level only. A scale from 1-5 has been revised for endosperm modification in maize. Score 1 is fully modified that my or may not be QPM, while as score of 5 means chalky, opaque and soft kernels. Breeders use a scale of 2-3 in early breeding generations but full modification is sought in later cycles in QPM converted back ground. The aim is to achieve complete grain modification without losing the *o2* phenotype.

At CIMMYT, a conservative approach was adopted in developing mo2 genotypes to strike a balance between proteins levels and grain quality and competetive yield levels. Therefore, emphasis was laid on maintaining its lysine content while making it competitive with conventional maize varieties. This improved *o2* maize was renamed as quality protein maize (QPM). QPM essentially has about twice the levels of lysine and tryptophan than normal maize and also increased levels of histidine, arginine, aspartic acid and glycine. It also has reduced levels of glutamic acid, alanine, leucine, especially, the lower levels of leucine is an added advantage as it results in a more balance leucinesoleucine ratio that helps to liberate more tryptophan.

In addition to these two genetic systems that is, o-2 and endosperm modifiers, a third genetic system consist of amino acid modifier genes affecting relative levels of lysine and tryptophan (Krivanek et al., 2007). As against the lysine and tryptophan levels of 2 and 0.4% in normal maize, the corresponding values in QPM are 4 and 0.8% respectively. This is largely due to a decrease in the zein fraction from 47.2% in normal maize to 22.8% in o2 mutants. Genes governing the levels of lysine have been mapped to the chromosome2, 4 and 7 (Wang et al., 2001; Wu et al., 2002). Such genes have been identified and include eEF1A (7L), eEF1A (4S), eEF1A (2S), FAA (1L), FAA (2S), FAA (2L), FAA (3S), FAA (4L), FAA (5L), FAA (7L), FAA (8S) and FAA (9S). It is now quite evident that from a simple genetic system in o-2, QPM has evolved by combination of various genetic systems, behaving to be handled like classical quantitative trait.

Breeding efforts and achievements: CIMMYTs role

Global research on QPM had faded elsewhere, however,

Table 1. CIMMYT gene pools/populations of QPM.

Pool/population	Adaptation	Maturity	Seed colour	Seed texture
Pool 15 QPM	Tropical	Early	White	Flint-dent
Pool 17 QPM	-do-	-do-	Yellow	Flint
Pool 18 QPM	-do-	-do-	-do-	Dent
Pool 23 QPM	-do-	Late	White	Flint
Pool 24 QPM	-do-	-do-	-do-	Dent
Pool 25 QPM	-do-	-do-	Yellow	Flint
Pool 26 QPM	-do-	-do-	-do-	Dent
Pool 27 QPM	Sub-tropical	Early	White	Flint-dent
Pool 29 QPM	-do-	-do-	Yellow	-do-
Pool 31 QPM	-do-	Medium	White	Flint
Pool 32 QPM	-do-	-do-	-do-	Dent
Pool 33 QPM	-do-	-do-	Yellow	Flint
Pool 34 QPM	Tropical	-do-	-do-	Dent
Population 61	Tropical	Early	Yellow	Flint
Population 62	-do-	-do-	White	Semi-flint
Population 63	-do-	-do-	White	Dent
Population 64	-do-	-do-	White	-do-
Population 65	-do-	-do-	Yellow	Flint
Population 66	-do-	-do-	-do-	Dent
Population 67	Sub-tropical	-do-	White	Flint/semi-flint
Population 68	-do-	-do-	-do-	Dent/semi dent
Population 69	-do-	Medium/late	Yellow	Flint
Population 70	-do-	-do-	-do-	Dent

Source : Vasal et al. 1993 a, b.

at a wide array of tropical and sub-tropical QPM gene pools and populations with hard endosperm have been developed (Bjarnson and Vasal, 1992). Initially the emphasis was on development of donor stock by selection for modified grain texture in QPM backgrounds using various selection schemes. The donor stocks were isolated from soft *o2* materials that showed vary-ing degrees of kernel modification due to differential accumulation of modifier genes. The development was accomplished by intra-population selection of genetic modifiers in *o2* background followed by grouping of modified *o2* sources into pools which were recombined. The materials from these two approaches were used as donor for conversion programmes. These efforts even though painstaking led to identification of four tropical hard endosperm populations (composite K; Ver 181-Ant gp venezula-1; Thai composite; PD 9MS6) and one highland (Composite-1). Later on, these donor stocks were used for large scale conversion of non-QPM materials into QPM into a wide array of genetic back-ground from different agro-climatic zones. Table 1 presents various germplasm pools and populations developed by CIMMYT for use in various national maize programmes. This resulted in release of a larger number of QPM varieties by conversion of elite tropical, subtropical and highland lines/populations into QPM versions. The yield gaps were progressively narrowed down to increase the acceptance environments (Table 2).

In 1985, when CIMMYT began its hybrid maize programme, there was corresponding shift of focus in case of QPM germplasm development from OPV's to hybrids for different target environments. Consequently, population improvement was replaced by hybrid breed-ing. Efforts were directed towards characterization of QPM germplasm in terms of combining ability and heterotic patterns through a serves of dialled studies world over. The result was release of a large number of QPM hybrids that out yielded the local non-QPM check in regional trails (Table 3).

Adoption and impact of QPM

The target countries for large scale cultivation of QPM have been those where maize finds substantial use for human consumption and animal feed. These countries have different levels of development ranging from deve-loped nations like Mexico and Brazil to developing /underdeveloped nations of Africa and Asia. Among humans, women and children have been major targets while as in case of animals, pig and poultry are major targets. In 1977, only four countries grew QPM but in 2003, more than 23 countries have released QPM varieties for large scale cultivation on area over 3.5 million hectares with Mexico alone accounting for about 2.5 million hectares. Presently, the area under QPM is about 2.5 million hectares. This has been largely possible due to the finding of QPM research at CIMMYT by NIPPON foundation Japan and Canadian International Development Agency (GDA). The Nippon funded project "The improvement of quality protein

Table 2. QPM varieties released since 1996.

Variety	Pedigree	Country
NB-nutrina	Poza Rica 8763	Nicaragua
Susma	Across 8363SR	Mozambique
Obtamapa	-do-	Mali
Nalongo	-do-	Uganda
Obatampa	-do-	Benin
BR-473	-do-	Brazil
BR-451	-do-	Brazil
Assume preto	-do-	Brazil
Obatampa	Across 8363SR	Burkina Faso
Obatampa	-do-	Guinea
VS-537C	Poza Rica 8763	Mexico
VS-538 C	Across 8762	Mexico
Espoir	-do-	Burkina faso
Obatampa	Across 8363SR	Cameroon
Mamaba	-do-	Guinea
CMS 475	-do-	Guinea
K 9101	-do-	Guinea
CMS 473	-do-	Guinea
WSQ104	Pool 15	Kenya
EV 99 QPM	-do-	Nigeria
EV 99 QPM	-do-	Senegal
DMRESR WQPM	-do-	Senegal
Susma	Across 8363SR	Senegal
Lishe-K1	Across 8363SR	Tanzania
Ev 99 QPM	-do-	Togo

Source : Cordova, 2000; Krivanek et al., 2007.

maize in selected developing countries", focussed on promotion of QPM in countries, where maize is a staple and where the probability of adoption and impact is high. In sub-saharan Africa, 17 countries are growing QPM on around 200000 hectares with Ghana alone accounting for about 70000 hectares, Obatampa being the major cultivar. NIPPON foundation, CIDA and Rockefeller foundation have been instrumental in promoting the development and dissemination of QPM in Africa (CIMMYT, 2005). The emphasis has been on conversion of elite maize OPV's and hybrids into QPM's. In India, QPM research was initiated under AICMIP in 1966 and resulted in three o2 composites namely Shakti, Rattan and Protina. Later on using endosperm modification system, mo- 2 composite "Shakti-1" was released in 1998. Later on under NATP, two hybrids "Shaktiman-1" and "Shaktiman-2" were also released using CIMMYT inbreds as parental lines (Prasanna, et al., 2001). In China, a number of high yielding QPM hybrids are under cultivation covering an area of about 1000 hectares. It is expected that by 2020, about 30% of maize area in China will be under QPM cultivars (Gill, 2008).

Impact dietary assessment studies

Most important target group for QPM consumption are children. Out of the 189 nations listed by UNICEF, Malawa, Mozambique, India and Bolivia have highest under five mortalities. The impact of QPM can be highly significant in such target group in these vulnerable nations. An early study on Indian children fed with food supplemented with normal maize, QPM and milk (Singh, et al., 1980). The results from children taking QPM were encouraging as the growth parameters recorded were comparable to those of milk. A more comprehensive study was carried out in Ghanaian children (0-15 months) given food supplemented with QPM and normal maize (Afriyiet et al., 1998). The QPM fed children were healthier, suffered fewer fatalities and had better growth rates. The impact of QPM in human nutrition will however, depend on a number of factors. It needs to be established whether the intake of QPM essentially results in enhanced protein utilization in children and adults. Moreover, the results have to be validated especially in the areas where malnutrition is prevalent and maize is an important component of diet.

Table 3. QPM hybrids released since 1996.

Hybrid	Pedigree	Country
HQINTA-993	(CMS-144 x CML-159) CML-176	Nicoragua
HB-PROTICTA	-do-	Guatemala
HQ-61	-do-	El-Savador
HQ-31	-do-	Honduras
Zhongdan 9409	Pool 33 x Temp QPM	China
Zhongdon 3850	-do-	China
QUIAN 2609	(Tai 19 x Tai 02) CML-171	China
Shaktiman-1	(CML-142 x CML-150) CMS -176	India
Shaktiman-2	CML-176 x CML-186	India
QS7705	-do-	South Africa
GH-132-28	P62 x P63	Ghana
BHQP-542	(CML-144 x CML-159) CML-176	Ethiopia
INIA	CML-161 x CML-165	Peru
FONAIAP	(CML-144 x CML-159) CML-176	Venezuala
HQ-2000	CML-161 x CML-165	Vietnam
H-441C	CML-186 x CML-142	Mexico
H-367C	CML-142 x CML-150	Mexico
H-553C	(CML + 42 x CML-150) CML-176	Mexico
H-519C	(CML-144 x CML-159) CML-176	Mexico
H-368C	CML-186 x CML-149	Mexico
H-469C	CML-176 x CML-186	Mexico
KH500Q	(CML-144 x CML-159) CML 1-81	Kenya
KH631Q	(CML + 44 x CML-159) CML-182	Kenya
Lishe-H1	(CML-4 x CML-159) CML-176	Tanzainia
Lishe-H2	Obatampa (CML-144 x CML-176	Tanziania
ZS261Q	(CZL01006 x CML-176 (CZLO1005 x CML-181)	Zimbabwe

Source : Cordova, 2000; Krivanek et al. 2007.

Another application of QPM is as animal feed, especially for monogastric animals such as pigs and poultry, which require a more complete protein than cereals alone can provide, as is case with normal maize that is deficient in lysine and tryptophan. A number of studies have proved that the more potential impact of QPM can be its use in commercial feeds for pigs and poultry as it results in improved growth. In Brazil and El-salvador, use of QPM in animal feed reduced use of soybean meal by about 50% besides reducing use of synthetic lysine substantially (Pereira, 1992). It also resulted in saving of 3-5% in development of feed for pig and poultry. In China, the QPM variety Zhong Dan-9409 has been used in animal feed. It has 8-15 per cent yield advantage and about 80 per cent more lysine and tryptophan. Zhai (2002) analysed the effect of replacing normal maize with QPM and found that in case of poultry, there was no apparent increase in amino acid digestibility but in case of pigs, there was a significantly higher amino acids and weight gain in case of pigs at various growth stages. Similarly, in Ghana, the QPM variety “Obatampa” has been used in both human nutrition as well as `animal feed. The potential impact of QPM as animal feed is restricted to Mexico, Brazil, China, Bolivia, which have high per capita meat supply. Contrarily, in African countries, due to the low meat supply market it a less potential marked for QPM as animal feed component

Genetic engineering for improving protein quality

Genetic engineering has targeted all the traits amenable to manipulation and quality is not exception to it. The use of genetic engineering in improving protein quality of maize is a very potential area of application of biotechnology since conventional breeding suffers from various draw backs. In fact the earlier breeding efforts have been some times frustrating with either no results or limited success. Grain protein quality is governed by various biochemical pathways and once those path-ways are elucidated, it is easier to manipulate them for bringing about desired changes in grain protein profile. Various biochemical pathways are potential targets for manipulation using genetic engineering (Shewry, 2007).

Increasing lysine rich proteins

Introduction of proteins that carry higher proportion of lysine residues is a unique approach to improve grain

protein. This approach offers advantages as it is not associated with any adverse effect on grain texture as in case of *o2* maturation. Furthermore, since a number of such proteins have been identified among cereals it will not attract too many regulatory controversies. A number of proteins have been found to have higher lysine content including β-amylase (5%), protein Z (7.1%), chrymotrypsin inhibitors CI-I (9.5%) and CI-2 (11.5%) and hordeothionin of barley. Yu et al. (2004) transformed maize with a lysine rich protein from potato pollen resulting in 50% increase in grain protein and lysine. But lack of information about its biological functions is a major concern for using it on large scale.

Reducing zein synthesis

In maize, RNA interference induced downregulation of 22 KDa α-zeins (Segal et al., 2003) and 19 KDa α-zeins (Huang et al., 2005) resulted in higher lysine content (upto 16-20% more lysine) much below the 02 (02) mutants. Double stranded RNA (ds RNA) had been used as a refined approach to simultaneously down regulate both 22 KDa and 19 KDa α -xeins resulting in increase in lysine from 2.83 to 5.62% and tryptophan from 0.69 to 1.22%. The advantage of this approach is based on the fact that the dominant nature of transgene ensures maintenance of quality in farmers fields under varying degrees of contamination fro pollen of normal maize, which is a major problem in case of recessive O2 mutants.

Increasing the level of free amino acids

Even though free acids account for small proportion of grain proteins, nevertheless they are also a potential target for genetic manipulation. The essential amino acids such as lysine, threonine and metheoine, in all higher plants, are synthesised from aspartic acid via a pathway that is highly branched and under complex feed back inhibition (Zhu et al., 2007). The key enzymes of pathway are aspartate kinase (AK) and dihydropicolinate synthase (DHPS).The former (AK) is important at early steps of pathway and is inhabited by both lysine and threonine while as latter is inhibited by lysine only. Mazur et al. (1999) expressed a *Corynebacterium* DHPS gene driven by globulin-1 promoter in aleurone and embryo of maize and observed 50-100% increase in free lysine. However, no increase in free lysine was observed when the same gene was expressed in endosperm (driven by glutelin-2 (-zein) promoter). Zhu and Galili (2003) expressed a becterial (*E. coli* or *Coryneoacterium*) feed back insensitive, DHPS in *Arabidopsis* and achieved 12 fold increase in grain lysine content. The expression of DHPS in maize increase the free lysine content in grain from 2 to 30% of total amino acid pool (Zhu et al., 2007). Monsanto in 2006 has also released transgenic maize with high lysive (lysine) by expressing feed back insensitive DHPS gene from *Corynebacterium* drive by globulin-1 gene promoter. The free lysive in grain (dry weight basis) increased from 2500-2800 ppm to 3500-5300 ppm.

Marker-assisted selection for QPM breeding

The development of QPM requires manipulation of various genetic systems such as O2, endosperm modifiers and amnic acid modifiers and as such conventional breeding procedures are quite laborious and the results some times frustrating. It is very tedious to conti-nuously select for optimum level of one trait while maintaining desired level of other. The conventional backcross strategy for conversion of normal maize to QPM suffers from two major problems. One that *o2* being a recessive trait, selection has to be carried out at each backcross in order to fix the recessive *o2* allele, prior to selection for endosperm modification, thereby extending the time period for line conversion. While as, latter is inhibited by lysine only. Moreover, the quality traits such as grain protein content cannot be selected prior to seed formation making screening very difficult. Besides, low cost and reliable methods of screening are not available.

Marker assisted selection is an appropriate techno-logy 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 se-quencing of maize genome being finished, a large number of market system are now available that are associated with *o2* and endosperm modification phenol-type (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. Both foreground MAS and background MAS can be effectively employed for selecting *o2* phenotype besides ensuring maximum recovery of recurrent parent. 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. reviewed enthusiasm in our endeavour to make QPM of real potential use."

Conclusion

QPM research has witnessed early enthusiasm, a phase of near abandonment and a phase of renewed interest. The key feature of all these phases has been the commitment of QPM breeders at CIMMYT and elsewhere and the results are quite obvious. Today improve (improved) QPM varieties and hybrids have been released in about 40 countries with African countries being the major target. CIMMYT has done all the good work to make QPM research a worthwhile

venture. It is in recognition of this commitment that S.K. Vasal (Maize breeder) and E. Villegas (Cereal chemist) were confessed upon the prestigious world food prize in 2000 for their efforts at CIMMYT to develop QPM. But the overall success of QPM has been due to painstaking efforts of a number of scientists at CIMMYT and elsewhere notably Magni Bjarnson, Kevin Pixley, Brain Larkins, Norman Borlaug, Hugo Cordovo, Timothy Reeves and S. Pandey, various agencies such as Nippon foundation, CIDA, Rockefeller foundation and Sasakann Global 2000 and various partner national maize research systems.

The most important goal of QPM research has been to reduce malnutrition in target countries through direct human consumption, even though, the impact, as of now, has now been as great perceived, it is expected that greater impact will accrue out of development and dissemination of improved hybrids, OPV's and Synthetics in Africa and Mesoamerica. The new QPM synthesis have desirable features such as low uniform ear placement, resistance to ear rot and root lodging and better grain protein profile (Cordovo, 2001) . Efforts are on at CIMMYT and elsewhere to transfer high lysine trait in elite stress tolerant genotypes through marker assisted selection. The sequencing of maize genome and identification of markers associated with protein and grain modification will help rapid identification of genes responsible for such traits and thereby accelerate the development of QPM varieties for target countries where probability of impact in high (Gibbon and Larkins, 2005). In terms of feed component, China, Brazil, Mexico and Vietnam are potential targets as maize is extensively used as component of animal feed.

For better dissemination and adoption of QPM in target countries we need to understand factors affecting is adoption/disadoption, agronomic characteristics, awareness about nutritional quality, availability of seed and ease of recycling (Kravinek et al., 2007). The words of S.K. Vasal are worthwhile to mention, "Good science is not free from difficulties, frustration and criticism. This should be viewed to generate creativity, revisiting different approaches and activities, and making constant adjustments for efficient use of resour-ces at all levels. Periodic reviews help to de-emphasize some aspects while expanding others, if necessary and introducing new initiatives, while were not in place already, therefore, it is very essential to make new initiatives, try novel ideas and pump reviewed enthusiasm in our endeavour to make QPM of real potential use."

REFERENCES

Prasanna B, Sarkar K (1991). Coordinate genetic regulation of maize endosperm. Maize genetics Perspectives ICAR pp. 74-86.

Bantle K, Prasanna B (2003). Simple sequence repeat polymorphium in QPM lines. Cuphytica 129: 337-344.

Babu R, Nair S, Kumar A, Venkatesh S, Shekhar J, Singh NN, Gupta H (2005). Two generation marker aided backcrossing for rapid conversion of normal maize lines to quality protein maize. Thero. Appl. Genet. 111: 888-897.

Bjarnson M, Vasal SK (1992). Breeding for quality protein maize. Plant Breed. Rev. 9: 181-216.

Cordova H (2001). Quality protein maize: improved nutrition and livelihoods for the poor. Maize Research Highlights 1999-2000, CIMMYT pp. 27-31.

CIMMYT (2005). The development and promotion of QPM in sub-saharan Africa, Progress Report Submitted to Neppon Foundation. 6: 312-324.

Damerval C, Devienee D (1993). Qualification of dominance for proteins plieotropically affected by opaque-2 in maize. Heredity 70: 38-51.

Danson J, Mbogori M, Kimani M, Lagat M, Kuria A, Diallo A (2006). Marker-assisted introgression of opaque 2 gene into herbicide tolerant elite maize inbred lines. African J. Biotech. 5: 2417-2422.

Gibbon B, Larkin B (2005). Molecular genetic approaches to developing quality protein maize. Trends Genet. 21: 227-233.

Geetha K, Lending C, Lopes M, Wallace J, Larkins B (1991). Opaque-2 modifiers increase α-zein synthesis and alter its spatial distribution in maize endosperm. Plant Cell 3: 1207-1219.

Gill G (2008). Quality protein maize and special purpose maize improvement. In "Recent Advances in crop improvement" CAS training at PAU from 05-25 Feb, 2008. pp. 377-385.

Huang S, Frizzi A, Florida C, Kruger D (2006). High lysine and high tryptophan transgenic maize resulting from reduction of both 19- and 22 KDa α-zeins. Plant Mol. Biol. 6: 525-535.

Huang S, Kruger D, Grizz A, Ordene R, Florida C, Adams W, Brown W,Luethy M (2005). High lysine corn produced by combination of enhanced lysine biosysnthesis and reduced zein accumulation. Plant Biotech. J. 3: 555-569.

Huang S, Adams W, Zhou Q, Malloy K, Voyles D, Anthony J, Kriz A, Luethy M (2004). Improving nutritional quality of maize proteins by expressing sense and antisense gents. J. Agric. Food Chem. 52: 1958-1964.

Krivanek A, Groote H, Gunaratna N, Diallo A, Freisen D (2007). Breeding and disseminating quality protein maize for Africa. Afr. J. Biotech. 6: 312-324.

Lauderdale J. (2002). Issues regarding targeting and adoption of quality protein maize. CIMMYT working paper p. 31.

Lopez M, Gloverson L, Larkins B (2004). Genetic mapping of opaque-2 modifier genes. Maize Genet. Newsletter 69: 165.

Mazur B, Krebbers E, Tingey S. (1999). Gene discovery and product development for grain quality traits. Sci. 285-372.

Prasanna B, Vasal S, Kasahun B, Singh N.N. (2001). Quality protein maize. Curr. Sci. 81: 1308-1319.

Paez A, Helm J, Zuber S (1969). Lysine content of opaque-2 kernels having different phenotypes. Crop Sci. 9: 251-252.

Segal G, Song R, Messing J (2003). A new opaque variant of maize by a single dominant RNA-interference – inducing transgene. Genet. 165: 387-397.

Segal G, Songh R, Messing J. (2003). A new opaque variant of maize by single dominant RNA-I inducing transgene. Genet. 165: 387-397.

Singh J, Koshy S, Agarwal K, Singh NN, Lodha M, Sethi A (1980). Relative efficiency of opaque-2 maize in growth of preschool children. Ind. J. Nutr. Dietet. 17: 326-334.

Singh NN, Venkatesh S (2006). Development of quality protein maize inbred lines. In: Heterosis in Crop Plants. Ed. Kaloo G, Rai M, Singh M, Kumar S. Res. Book Center, New Delhi, pp. 102-113.

Shewry P (2007). Improving protein content and composition of cereal grain. J. Cereal Sci. 46: 239-250.

Shewry P, Halford N (2002). Cereal seed storage proteins : structure, properties and role in grain utilisation, J. Expt. Bot. 53: 947-958.

Toro A, Medici L, Sodek L, Lea P, Azevedo R (2003). Distribution of soluble amino-acids in maize endosperm mutants. *Scientia Agricola* 60: 91-96.

Vasal SK (2001). Quality protein maize development : An exciting experience. Seventh Eastern and South Africa Regional Maize Conference pp. 3-6.

Vasal SK, Srinivasan G, Pandey S, Ganzalez F, Crossa J, Beck DF (1993a). Heterosis and combinign abiity of CIMMYT's Quality protein maize germplasm. I. lowland tropical. Crop Sci. 33: 46-51.

Vasal SK, Srinwasan G, Gonzalez C, Beck D, Crossa J (1993b). Heterosis and combining ability of CIMMYT's quality protein maize germplasm. II. Sub-tropical. Crop Sci. 33: 51-57.

Vasal SK (2000). Quality protein maize story. Proceedings of workshop on Improving Human nutrition through agriculture. The Role of International Agricultural Research, IRRI. pp. 1-16.

Wang X, Woo Y, Kim C, Larkins B (2001). Quantitative trait locus mapping of loci influencing elongation factor 1 alpha content in maize endosperm. Plant Physiol. 125: 1271-1282.

Wu R, Lou X, Ma C, Wang X, Larkins B, Casella G (2002). An improved genetic model generates high resolution mapping of QTL for protein quality in maize. PNAS 99: 11281-11286.

Yu J, Peng P, Zhang X, Zhao Q, Zhy D, Su X, Liu J, Ao G (2004). Seed specific expression of lysine rich protein Sb401 gene significantly increases both lysine and total protein in maize seeds. Mol. Breed. 14: *1-7.*

Zhai S (2002). Nutritional evaluation and utilization of QPM Zhang Dan 9404 in laying ren feeds. M.Sc. Thesis. North Western Agri. & Forestry Univ. of Sci. & Tech, China, pp. 13-21.

Zhu X, Galali G (2003). Increased lysine synthesis coupled with a knock out of its catabolism synergistically boosts lysine content an also trans regulators metabolism for other amino acids. Plant Cell 15: 845-853.

Zhu C, Naqvi S, Sonia G, Pelacho A, Capell T, Christou P (2007). Transgenic strategies for nutritional enhancement of plant. Trends Plant Sci. 12: 548-555.