

# Role of quality protein maize in nutritional security as food and feed

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## Abstract

Quality protein maize (QPM) nutrient evaluation proved that, it is superior over non- QPM cultivars for utilization of live stocks and human beings. Zeins are the main protein component of seed stores in maize.  $\alpha$ ,  $\beta$ ,  $\gamma$ , and  $\delta$  are sub families of the zeins. In these  $\alpha$ zeins are important prolamin sub unit in maize. Quality protein maize cultivars having hard kernel and high amount of lysine & tryptophan are due to combination of *o2* (*opaque-2*) allele with genetic modifiers possessed by breeding. QPM varieties have double amount of lysine and tryptophan than normal maize varieties. QPM helps in the reduction of malnutrition problem in the humans and increase the live stocks, poultry production.

**Key words:** - Quality protein maize, *opaque 2*, Nutrition benefits of QPM, Essential amino acids.

## Introduction

Maize (*Zea mays* L.) is the queen of the cereal and 3<sup>rd</sup> important cereal crop after wheat and rice. It is used for consumption of human beings and livestock feed (Prasanna et al., 2001). Worldwide 15% protein & 19% calories are derived from maize. In developed countries 78 percent of total maize products are utilized as feed of livestock (Sofi et al., 2009). African people consuming 17-60% of protein and 1/5<sup>th</sup> daily calories are supplied by maize products. The objective of this review was to determine the dietary effects of the substituting normal maize meal with Quality protein maize meal on the growth performance, feed utilization of broiler chicken and human. Malnutrition is one of the widely spread problem throughout the world. It is solved by the significant development and technological advance in the agriculture (Neeraja et al., 2017). Micro nutrient deficiency in staple food diet causes the hidden hunger. Highly developing and less developed countries are affected by the hidden hunger (Bouis and Saltzman 2017). Almost 2 billion people are under micronutrient deficiency and 815 million people are under nourished problems. One or more types of malnutrition are faced by 88 percent of countries and 45 percent of children under the age of five years is due to the deficiency of micronutrients (Black et al., 2013). For growth and development of human beings balanced nutrition are required containing essential amino acids, minerals and vitamins (Bouis et al., 2011). Protein energy malnutrition causes poor intellectual development and physical function disorders leading to mortality. Marasmus and Kwashiorkor are the major manifestation of protein energy malnutrition. Marasmus is caused due to insufficient calories & proteins while protein deficiency causes Kwashiorkor (Bain et al.,

2013). Maize consumption is differing from country to country. Due to lack of essential amino acids like tryptophan and lysine its make the maize protein with low nutritional significance to the humans. In maize endosperm lysine is the most limiting amino acid than tryptophan (Prasanna et al., 2001). QPM products are used as a supplementary food for pregnant women, lactating mothers and young children globally.

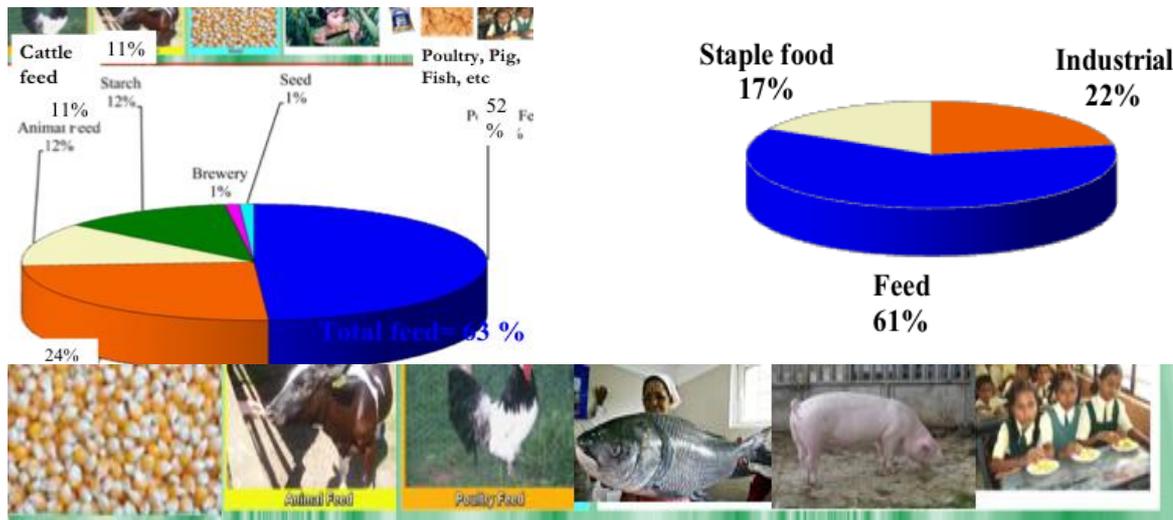


Figure: - 1 Current Maize Utilization in India & Global

**Maize kernel structure:-**

Maize kernel consist of 3 parts i.e. pericarp, embryo and endosperm at the level of 6, 12 & 82%. The outer most layer covering the kernel that preserves and protects the nutrient value inner side is known as pericarp. Embryo axis and the scutellum are composed by mature embryo. Protein is present in both endosperm and embryo. But superior quantity as well as quality protein are germ proteins. Generally endosperm contains starchy, endosperm, aleurone layer and basal transfer layer (fig 2).

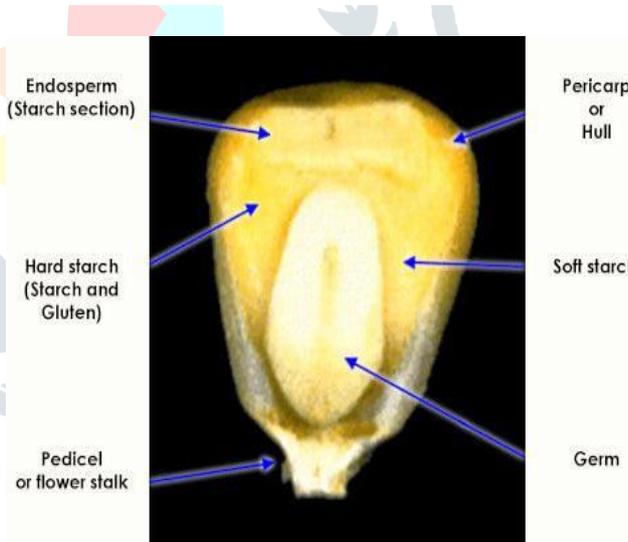


Figure: - 2 Maize kernel structure

Specialized hydrolytic enzymes rich cell make the aleurone outer most layer and it has starchy and vitreous regions in endosperm. Insoluble accretions which are present in the vitreous region forms the zein protein bodies in the lumen of the RER (rough endoplasmic reticulum) towards maturity it is densely packed (Gibbon, B. C and Larkins, B. A., 2005). Mainly four types of constitute up to 50-70% of endosperm of maize and those are rich in glutamine, leucine and proline and poor in tryptophan and lysine. Generally zeins are prolamins of maize grains solvable in alcohol mainly  $\alpha$ -zeins highly and little bit of  $\beta$ ,  $\gamma$ , and  $\delta$  also. The non- zein protein fraction is balanced and rich in lysine and tryptophan (Vasal, S. K., 2002).

### Mutants of Quality Protein Maize:-

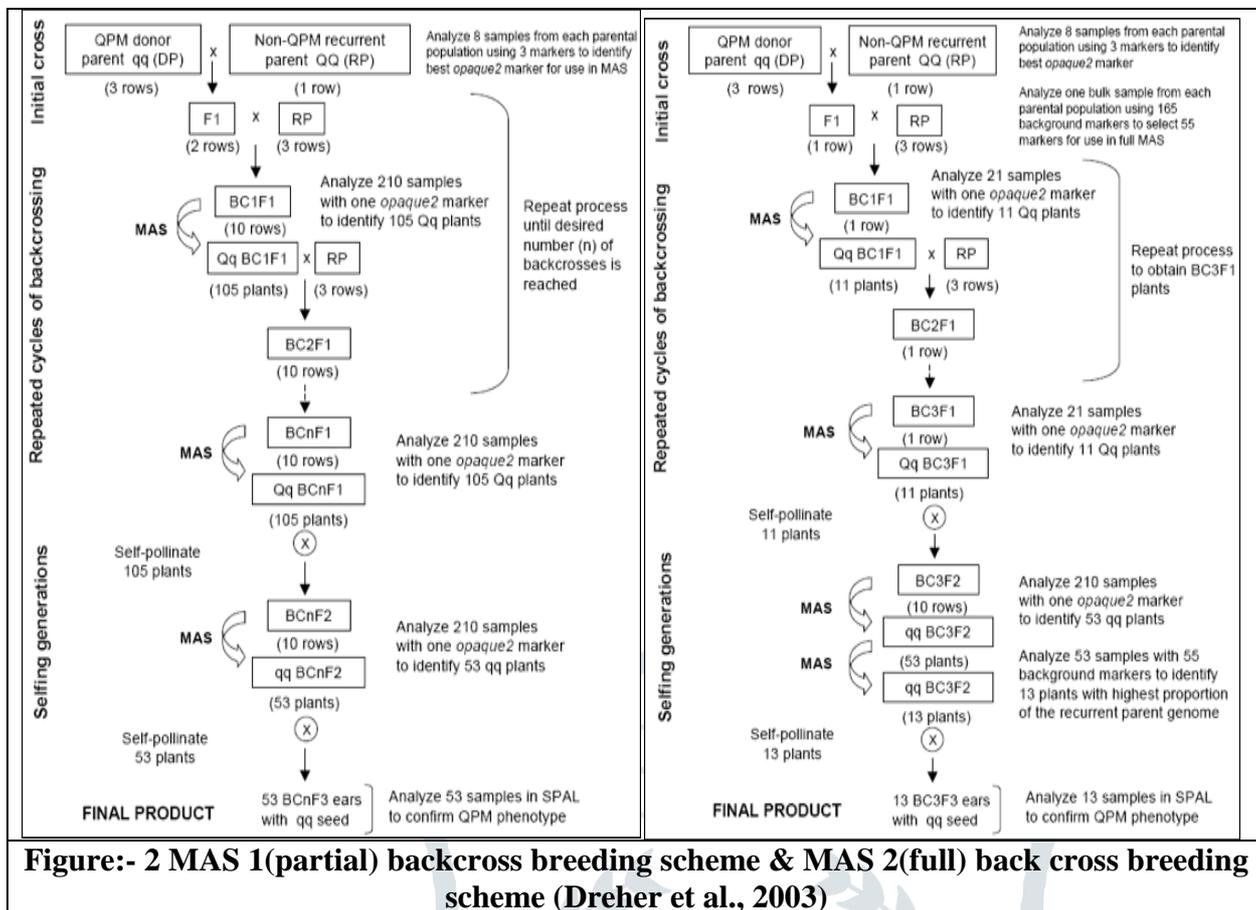
Higher lysine and tryptophan maize mutants were identified in the 1960&1970s i.e. *o2* (*opaque-2*), *fl2* (*floury-2*), *o7* (*opaque*), *o6* (*opaque-6*) and *fl3* (*floury-3*).

Year of discovery	Researchers	Gene	Allele
1964	Mertz, Bates & Nelson	<i>opaque-2</i>	<i>o2</i>
1965	Nelson, Mertz & Bates	<i>floury-2</i>	<i>fl2</i>
1971	Mc White	<i>opaque-6</i>	<i>o6</i>
1975	Ma & Nelson	<i>opaque-7</i>	<i>o7</i>
1975	Ma & Nelson	<i>floury-3</i>	<i>fl3</i>

- Recessive (*o2*) mutation of maize homozygous are having higher lysine and tryptophan content than dominant (*O2O2*) or heterozygous (*O2o2*) for the *opaque-2* locus (*o2o2*).
- For the development of high lysine and tryptophan in the maize genetic manipulation in *o2* mutation is required.

### Marker-assisted breeding in QPM

Marker assisted selection in QPM is necessary because every back cross generation needs selfing to identify the *o2* recessive genes and at least 6 BC (Back cross) generation are required to get satisfactory level of the recurrent parent genome. For maintaining homozygous *o2* gene multiple modifiers must be selected followed by identification of lysine and tryptophan level increment in every breeding generation through biochemical test. Sequencing of the maize genome indicated that *opaque-2* & endosperm modification phenotype might be associated with each other (Lopez et al., 2004, Bante, K and Prasanna, B. M. 2003). A convenient utilization of such markers will greatly enhance the efficacy of selection for improvement of grain protein in maize and also reduces the cost and time. MAS used for development of QPM parental lines and development of QPM hybrid require less than half the time through conventional breeding. For the introgression of *o2* gene into elite maize inbred lines for rapid BC conversion programme different markers are used. Use of markers QPM and endosperm modification can enormously develop the modified kernel in the background of the quality protein maize isolating through effective selection (Singh et al., 2017).



**Figure:- 2 MAS 1(partial) backcross breeding scheme & MAS 2(full) back cross breeding scheme (Dreher et al., 2003)**

**Molecular approaches**

Table 1. QPM breeding investigation by using different molecular methods

Marker	Plant material	Aim	Reference
SSR	Non QPM & QPM	Marker assisted introgression of the o2 trait	Manna et al., 2005
ISSR primers and variety diagnostic markers	Non QPM & QPM	Molecular breeding program development	Nikongolo et al., 2015
SDS-Page	QPM grain	Protein identification	Zhang et al., 2015 Zhou et al., 2016
Proteomic analysis	QPM inbred lines	Identification of proteins associated with tolerance to combined drought and heat stress	Pfunde 2016
SNP markers	QPM lines	Genetic diversity studies	Pfunde 2016
SNP based genetic distance	QPM inbred lines	Heterotic grouping	Badu-Apraku et al., 2015

**Table 2.CIMMYT QPM gene pools and their characteristics (Prasanna et al., 2001)**

QPM Pool no.	Adaptation	Maturity	Seed colour	Seed texture	Kernel quality characteristics			Quality index
					Protein %	lysine%	Tryptophan%	
Pool 15 QPM	Tropical	Early	White	Flint-Dent	9.1	4.2	0.94	4.6
<b>Pool 17 QPM</b>	<b>Tropical</b>	<b>Early</b>	<b>Yellow</b>	<b>Flint</b>	<b>8.9</b>	<b>4.5</b>	<b>1.04</b>	<b>4.5</b>
Pool 18 QPM	Tropical	Early	Yellow	Dent	9.9	4.0	0.93	4.6
Pool 23 QPM	Tropical	Late	White	Flint	9.1	3.8	1.03	4.2
Pool 24 QPM	Tropical	Late	White	Dent	9.4	3.8	0.92	4.0
Pool 25 QPM	Tropical	Late	Yellow	Flint	9.8	4.0	0.94	4.0
Pool 26 QPM	Tropical	Late	Yellow	Dent	9.5	4.1	0.90	4.3
<b>Pool 27 QPM</b>	<b>Subtropical</b>	<b>Early</b>	<b>White</b>	<b>Flint-Dent</b>	<b>9.5</b>	<b>4.2</b>	<b>1.05</b>	<b>4.8</b>
<b>Pool 29 QPM</b>	<b>Subtropical</b>	<b>Early</b>	<b>Yellow</b>	<b>Flint-Dent</b>	<b>9.2</b>	<b>4.3</b>	<b>1.06</b>	<b>4.8</b>
<b>Pool 31 QPM</b>	<b>Subtropical</b>	<b>Medium</b>	<b>White</b>	<b>Flint</b>	<b>10.2</b>	<b>4.1</b>	<b>0.96</b>	<b>4.5</b>
Pool 32 QPM	Subtropical	Medium	White	Dent	8.9	4.2	1.04	4.5
<b>Pool 33 QPM</b>	<b>Subtropical</b>	<b>Medium</b>	<b>Yellow</b>	<b>Flint</b>	<b>9.3</b>	<b>-</b>	<b>1.05</b>	<b>4.2</b>
<b>Pool 34 QPM</b>	<b>Subtropical</b>	<b>Medium</b>	<b>Yellow</b>	<b>Dent</b>	<b>9.1</b>	<b>4.1</b>	<b>1.10</b>	<b>4.5</b>

### Essential Amino Acids importance

Proper development and growth of human body require 0.66 g protein per kg of body weight (WHO 2007). 20% of human body is made up of protein which plays important role in almost all biological process. Nearly 20 amino acids are required and normally merge into protein (Lea and Azevedo 2003). Out of 20 amino acids 9 are not synthesized by humans and monogastric animals like poultry and are supplied by the diet only (Galili et al., 2002). Limited availability of lysine/ tryptophan affects the use of other amino acids by the body. Its limitation causes the growth delay, skeletal impaired development and aberrant behavior (Moehn et al 2004).

### Animal nutrition

In order to reduce use of synthetic lysine (Burgoon et al., 1992), the focus should be on use quality protein maize as a substituted to maintain nutritional balance in poultry and pig feeds.



**Figure: -3 Differences between QPM & normal maize fed**

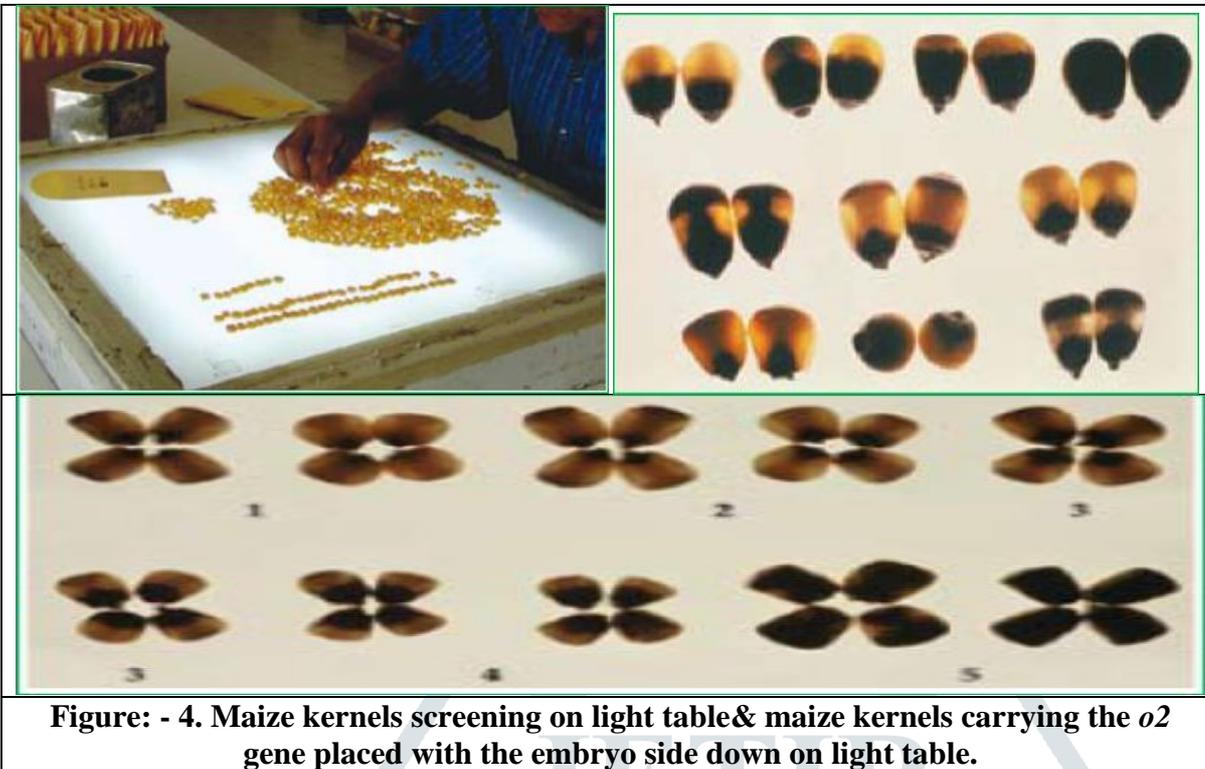
**Genetics of high lysine and tryptophan maize:** - Lysine and tryptophan improvement in the maize includes manipulating of the 3 different genetic systems. It includes simple recessive allele of the *o2* gene, which should be present in homozygous recessive condition. Zein is abundant protein in the grain endosperm.  $\alpha$  zein is mainly poor in lysine and tryptophan consist (Moro, G. L et al., 1995). Homozygous *o2* mutant causes the decrease in the production of the  $\alpha$  zein fraction of endosperm protein and equally increase in the fraction of non-zein proteins that naturally consist of higher level of the lysine and tryptophan (Prasanna et al., 2001). Level of the lysine and tryptophan is confirmed by the endosperm present in the *o2*. It contain little bit modified loci that influences endosperm lysine and tryptophan levels. *O2* genes modify the soft endosperm in to hard endosperm. Gamma zeins plays important role in the hard endosperm phenotype retention, resulting in *o2* modified hard endosperm grains having nearly twice amount of the gamma zein in the endosperm when compare to the particular *o2* mutants (Wallace et al., 1990). RNA interference technology is highly used to knocked down 27 & 16k Da  $\gamma$ -zein which are conserved in the sequence of DNA. It helps in rectifying the role of gamma zein in the endosperm hardness (Holding and Messing., 2010). For *o2* modifier gene two different types of the QTLs are identified. One is associated with increased expression and second is *o15* linked at different chromosome which causes decrement in expression of 27kDa  $\gamma$ -zein (Dannenhoffer et al., 1995). Elimination of  $\gamma$ -zeins obstructs endosperm modification by *o2* modifiers. Partial opacity occurs when the 27 and 16kDa  $\gamma$ -zeins are knocked-down by  $\gamma$  RNAi. It was strongly intensified that when the  $\gamma$  RNAi and  $\beta$  RNAi both were combined opacity is caused by an incomplete embedding of starch granules in the vitreous area not by reducing the thickness of the vitreous endosperms. Because the expression of the  $\beta$ -zein gene is also regulated by *o2* and insignificantly reduced in QPM, the amount of  $\gamma$ -zeins would become critical to keep starch granules embedded in the vitreous area.

### Identification of QPM genotype:

Generally the only effective approach at present is to physically screen the kernels using a light table for identification of promising QPM genotypes with desirable kernel modification attributes. In order to differentiate hard endosperm maize type from soft *o2o2* genotypes a light table box is used.

**Principle behind light table selection:-** Genotypes *o2o2* carry an undesirable characteristics i.e. kernel softness, which on light table is seen as complete opaqueness. Segregation of genes for endosperm hardness express varying degrees of softness/ hardness in the endosperm of segregating generation. *O2O2* genotype is hard and has desirable kernel characteristic and is therefore translucent. Degree of opaqueness is used to identify kernels with the *o2o2* genotype in light table. Variation in classes should be scored 1-5.

- 1- denotes 100 percent not *opaque*
- 2- denotes 25 percent *opaque*
- 3- denotes 50 percent *opaque*
- 4- denotes 75 percent *opaque*
- 5- denotes 100 percent *opaque*



**Figure: - 4. Maize kernels screening on light table & maize kernels carrying the *o2* gene placed with the embryo side down on light table.**

#### QPM Nutritional benefits:

QPM feeding trails are conducted on nutrient deficient children by giving QPM as only protein sources and it gives the same results when compared with modified cow milk taken children in normal diet (Graham et al., 1990). Guatemala states that *o2* maize has 90 percent of milk protein as compare to 40 percent in normal maize (Prasanna et al., 2001). QPM contain less leucine which helps in the high tryptophan liberating for niacin biosynthesis even if QPM and normal maize having niacin at same level. The QPM reduces the pellagra (Vasal 2001).

In animal feed many studies are conducted to assess the nutritional benefits and QPM biological superiority. It was 1<sup>st</sup> conducted on rats. Threefold increase in development rate was observed when fed with 90% QPM (Mertz et al., 1964). Doubled weight gain was observed in pigs when fed with QPM than normal (Burgoon et al., 1992). In poultry also broilers growth and development increased with the use of QPM feed (Onimisi et al., 2008).

**Table: - 4 Quality protein maize cultivars released for commercial cultivation in India (Gupta et al., 2009)**

Pedigree	Year of release	Varieties	Maturity group	Center	Protein (%)	Tryptophan (%)
Composite	1970	Shakti	Full season	AICRP	10.6	0.88
Composite	1970	Rattana	Full season	AICRP	10.0	0.90
Composite	1970	Protina	Full season	AICRP	11.0	0.97
Composite	1997	Shakti-1	Full season	DMR	9.30	0.95
(CML142*CML150)*CML186	2001	Shaktiman-1	Full season	Dholi	9.60	1.01

CML 176*CML 186	2004	Shaktiman-2	Full season	Dholi	9.30	1.04
HKI 193-1*HKI 163	2005	HQPM-1	Full season	Uchani	9.36	0.94
CML 161*CML 163	2006	Shaktiman-3	Full season	Dholi	9.63	0.73
CML1161*CML 169	2006	Shaktiman-4	Full season	Dholi	9.98	0.93
HKI 163*HKI 161	2007	HQPM-5	Full season	Dholi	9.80	0.76
HKI 193-1*HKI 161	2008	HQPM-7	Full season	Uchani	9.42	0.72
VQL-1*VQL-2	2008	Vivek QPM-9	Extra early	Almora	8.46	0.83

### List of promising orange QPM lines (ICAR-India Institute of Maize Research)

Sr.No.	Inbred Line	Opaqueness (%)	Protein (%)	Tryptophan (%)
1	DQL 2300	100	7.92	0.69
2	DQL 2029-1	50-75	7.09	0.98
3	DQL 2323	50-75	8.42	0.97
4	DQL 2104	50-75	8.24	0.89
5	DQL 2322	25-50	10.11	0.92
6	DQL 2034-1	25	10.64	0.92
7	DQL 2015-1	25	10.21	0.84
8	DQL 2082	25	8.42	0.84
9	DQL 2305	25	7.99	0.79
10	DQL 2081	25-50	7.08	0.96
11	DQL 2333	25	7.21	0.78
12	DQL 2016-1	25	10.46	0.77
13	DQL 2068	25	8.72	0.73
14	DQL 2262	25	8.11	0.7
15	DQL 2238	<25	8.11	0.69
16	DQL 2240	25	7.76	0.67
17	DQL 2244	25	7.91	0.67
18	DQL 2306	50	7.72	0.65

### List of promising white QPM lines (ICAR-India Institute of Maize Research)

S.NO	Inbred Line	Opaqueness(%)	Protein(%)	Tryptophan(%)
1	DQL 32-1-1-2-3-1	75-100	8.56	0.90
2	DQL 111-1-1-2-1	25	7.84	0.82
3	DQL 218-1-1-1	25	8.63	0.68
4	DQL 205-1-3-5	25	8.32	0.66
5	DQL 32-1-1-2-1	25	8.63	0.66
6	DQL 267-1-2-3-1	25	8.29	0.64
7	DQL 295-1-1-1	25	8.48	0.62
8	DQL 110-1-1-1	25	7.93	0.61
9	DQL 2003	25	8.33	0.60
10	DQL 2007	25	7.74	0.60

### Conclusion

Maize is hugely growing cereal crop in the world which plays significant role in the nutrition of humans as well as live stocks. Maize is the promising crop to meet the demands of the consumer through high quality

and nutritious food. Poor people who use QPM as staple food get more nutritional security. Malnutrition deficiency in children, pregnant women and old people can be solved and quickly recovered by the quality protein maize.

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