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Improvement of Quality Protein Maize

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Introduction:

Quality protein maize (QPM) originally developed in the late 1990s at CIMMYT, Mexico possesses roughly twice as much usable protein as normal maize grown in the tropics. The improved quality of the protein in QPM is due to enhancement in lysine and tryptophan the two limiting amino acids that are known to be regulated by *opaque*2 gene and associated modifiers.QPM has widely been adopted for cultivation in the developing world to fight protein malnutrition (Vasal *et al.* 1980). In India, QPM was released for commercial cultivation almost a decade ago by introducing QPM lines from CIMMYT. However, all these inbred lines are of longer duration and thus, give rise to QPM hybrids of full season maturity (Yadav, 2001). Utilizing marker assisted selection we transferred *opaque*2, a recessive gene, to two early maturing Indian inbreds that were, in turn, crossed to give rise to an early duration QPM hybrid, Vivek QPM 9, with 30% higher lysine and 40% more tryptophan while retaining the same level of productivity (Babu *et al.* 2005). Vivek QPM 9 yielded at par with Vivek Maize Hybrid 9 in the multi-location yield trials (Anonymous 2008).

Quality Protein Maize

It is an improved variety of maize which contains higher amount of lysine and tryptophan with lower amount of Leucine and Iso-Leucine in the endosperm than those contained in normal maize. Such balanced combination of amino acids in the endosperm results into its higher biological value ensuring more availability of protein to human and animal than normal maize or even all cereals and pulses.

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Need of Quality Protein Maize

- Significant advances have been made in genetic enhancement of crop plants for nutritional value.
- However, malnutrition still remains a widespread problem, and is particularly severe in developing countries with low per capita income. Several million people, particularly in the developing countries, derive their protein and calorie requirements from maize Animal protein, of course being of higher quality, is scarce and expensive, thereby unavailable to a vast sector of the population.
- Maize is a major cereal crop and plays very important role in human and animal nutrition in a number of developed and developing countries, worldwide.
- With its high content of carbohydrates, fats, proteins, some of the important vitamins and minerals, maize acquired a well-deserved reputation as a "poor man's nutria-cereal"

Nutritional Limitations of Maize

1. Low lysine and tryptophan which is an essential amino acids and lacking in *Zein* proteins, it is Major seed protein (60%), Lysine level is about 2% (4% recommended by FAO).
2. Low availability of niacin. Need to improve nutrition for humans and animals.

Maize in developing countries

1. Primarily animal feed in -: East and Southeast Asia and primarily human food in Africa, Central America and South Asia.
2. 15-56% of total daily calories for several hundred million people including weaning children in 25 count.
3. Demand by 2021will double the 1995 level and surpass the demand for wheat and rice.
4. Supply will be by 10% from international trade and 90% must be grown domestically.

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Opaque2 – a gene for improving quality of protein in Maize

- A natural spontaneous maize mutant with soft and opaque grain was found in a maize field in USA during the 1920s which was later named as *opaque2* (*o2*) maize by Singleton.
- The mutant was passed onto Mertz at Purdue University, USA, who, in turn, reported that the *o2* homozygous maize contained substantially higher lysine (+69%) in the grain endosperm compared to normal maize.

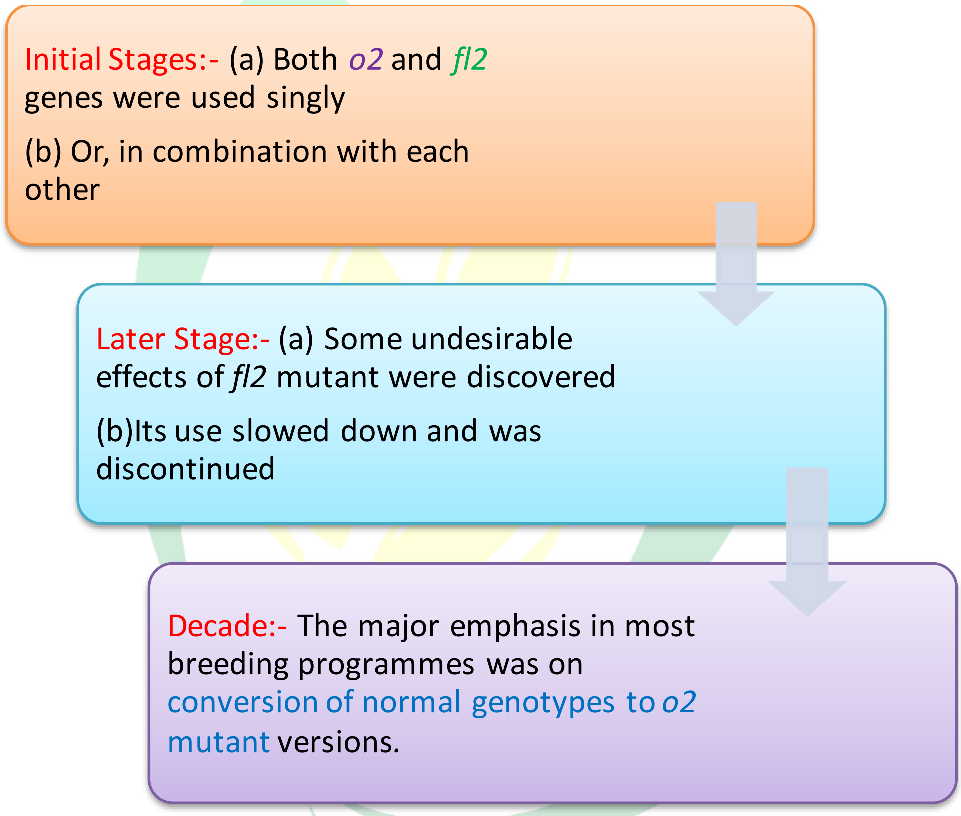

Fig 01: Boom Bust representation of *o2* and *fl2 genes in breeding programs*

- By increase in lysine content biological value of the *o2* maize protein is doubled and this increment in quality of protein is due to increase in the ratio of non-zein to zein proteins.
- For liberating more tryptophan from biosynthesis of niacin further, reduction of leucine in these mutants is considered desirable as it makes the leucine–isoleucine ratio more balanced.

- Although the *o2* mutant had better lysine content, the pleiotropic effects of this gene reported in the form of soft endosperm, increased susceptibility to insect-pests and fungal diseases, inferior food processing and reduction in grain yield, which discouraged its acceptability.
- Initiation of Breeding programmes to develop inbred lines by using Populations with Endosperm quality mutants, mainly *o2*.

Early efforts and experiences in using *o2* cultivars

o2 direct utilization in breeding programmes resulted in

1. Soft endosperm
2. Damaged kernels
3. Susceptibility to pests and fungal diseases
4. Reduced yields
5. Inferior food processing

Nutritional superiority of QPM: A compression

Protein intake utilization of:

- Common maize - 37%
- *o2* maize protein -74%

Maize required for nitrogen equilibrium per kg of body weight

- Normal maize -24 g
- QPM-8 g

Other nutritional benefits of QPM

- Higher tryptophan and lower leucine content,
- Higher calcium and carbohydrate and carotene
- Higher niacin

Nutritional superiority of QPM: A compression

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Table 01: Nutrional comparison Normal versus QPM

Nutrional comparission Normal versus QPM		
	Normal	QPM
Lycine*	160-180	256-300
Tryptophan*	30-40	60-100
Leucine *	827	507
Isoleucine*	206	193
True protein digestibility+	82	92
Biological value+	45	80

Development of QPM genotypes through conventional breeding

- Development of Acceptable QPM lines by combining the nutritional advantages of *o2* mutation with the *o2* modifiers.
- During the 1980s, CIMMYT took initiatives to convert a number of non-QPM genotypes to QPM genotypes, they followed a 'modified backcrossing-cum-recurrent selection'.
- During the conversion process, they also emphasized grain yield, kernel modification, reduced ear rot incidence and other agronomic traits.
- In a short span of 5–6 years, CIMMYT could convert many normal germplasm into QPM.
- Two scientists of CIMMYT, Mexico, Dr. S. K. Vasal and Dr. Evangelina Villegas, for a period of three decades led to development of Quality Protein Maize (QPM) with hard kernel, good taste and other consumer favouring characteristics.
- QPM research and development spread from Mexico to Central and South America, Africa, Europe and Asia.
- Awarded 2000 "World food prize" for path breaking research.1970 -India is one of the first few countries to focus on *o2* maize and released three o2 composites, namely Shakti, Rattan and Protina
- 1997- modified superior *o2* composite 'Shakti 1'.

- Later, India released eight QPM hybrids, seven of which were developed from the QPM inbreds of CIMMYT as parental lines and are of full season maturity.(HQPM1, HQPM5 ,HQPM 7 Vivek QPM 9)

Genetic systems and their role in enhancing the level of limiting amino acids in QPM

Development of QPM involves three genetic systems, *viz.*

- Recessive homozygous allele of the *o*2 gene
- Modifiers for kernel hardness
- Amino acid modifiers

(I) Recessive homozygous allele of the *o2* gene

- Central component of the QPM.
- Gene codes a transcription factor, a regulator for zein synthesis.
- α-zeins, are the most abundant proteins in the maize endosperm.
- They are known to be poor in amino acids like tryptophan and lysine.
- The o2 allele in homozygous condition reduces production of alpha-zeins and triggers increase in the level of lysine and tryptophan. Involved in the synthesis of the enzyme that is associated with free lysine degradation. Reduction in this enzyme leads to a corresponding increase in free lysine in the endosperm.

(II) Modifiers for kernel hardness

- Increased level of the gamma-zein is likely to contribute to the recovery of hard endosperm.*o2* modified (QPM) grains have double the amount of gamma-zeins in the endosperm compared to the *o2* mutants Two genes responsible for the grain hardness, mapped to the long arm of chromosome 7 and one of them is located near the gamma- zein gene '*gzr 1'*.
- It also affects the relative level of lysine and tryptophan content in grain endosperm.

Table 02: QPM cultivars released for commercial cultivation in India

Name	Pedigree	Release Year	Maturity group	Centre's name
Shakti	Composite	1970	Full season	AICRP
Rattan	Composite	1970	Full season	AICRP
Protina	Composite	1970	Full season	AICRP
Shakti 1	Composite	1997	Full season	DMR
Shaktiman 2	CML 176 x CML 186	2004	Full season	Dholi
HQPM 1	HKI 193-1 x HKI 163	2005	Full season	Uchani
Shaktiman 3	CML 161 x CML 163	2006	Full season	Dholi
Shaktiman 4	CML 161 x CML 169	2006	Full season	Dholi
Vivek QPM 9	VQL 1 x VQL 2	2008	Extra early	Almora

Development of QPM hybrid through marker assisted selection: Conversion of normal Maize inbreds into QPM

- In order to shorten the period required for development of QPM hybrids through the conventional method of backcrossing, marker-assisted selection (MAS) can be used.
- Few molecular markers were already known within the *o2* gene and these makers were capable of detecting the *o2* gene even in hetero-zygous state.
- To convert normal maize hybrid into QPM hybrid, a promising hybrid *viz.* Vivek Maize Hybrid 9 (developed by Vivekananda Parvatiya Krishi Anusandhan Sansthan, Almora) was selected for converting into QPM.

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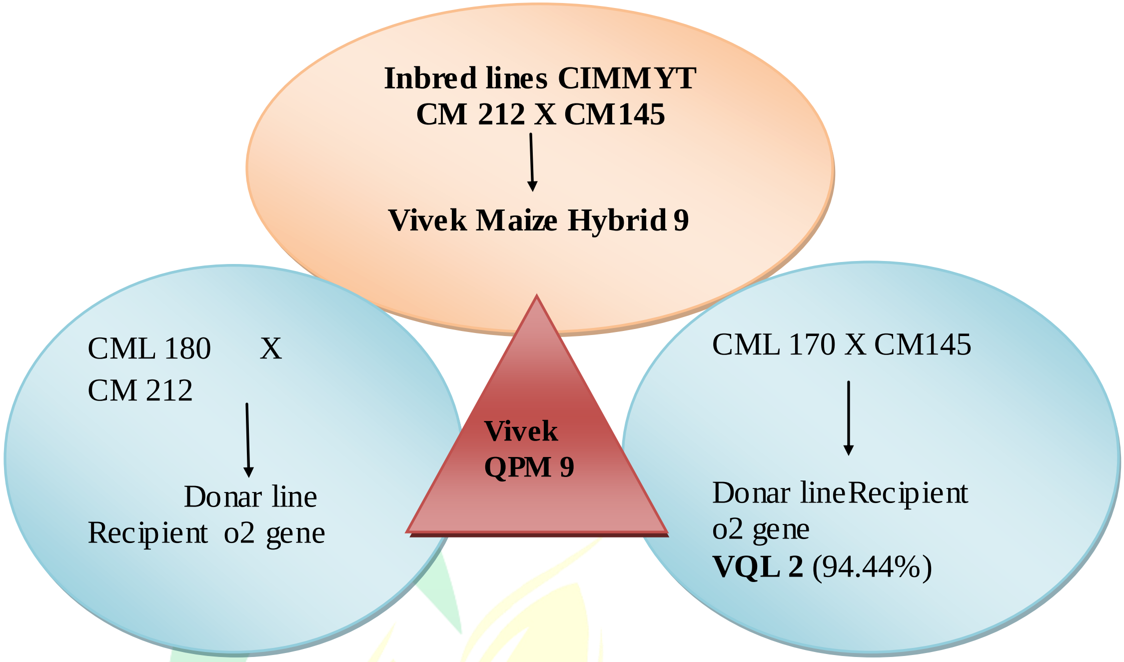

Fig 02: Development of QPM hybrid through marker assisted selection: Conversion of normal maize inbreds into QPM

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- The hybrid was released for commercial cultivation in Himalayan states, Andhra Pradesh, Tamil Nadu, Karnataka and Maharashtra by the Central Seed Sub-committee on Crop Standard and Notification in the year 2000.
- Kernel hardness was monitored through light box and the level of tryptophan was measured through biochemical method following Babu *et al.* 2005 and lysine by calorimetric analysis or by indirectly inferring lysine content through colorimetric analysis of tryptophan content.(Villegas *et al.* 1984)

- Danson *et al.* 2006, who deployed MAS to intrigues o2 gene for developing QPM inbred lines at CIMMYT. We recovered inbred lines homozygous for o2 gene with more than 90–95% recurrent parent genome coupled with 6–24% higher tryptophan within a short span of three years.

Table 03: Comparison of QPM, non-QPM inbreds and hybrids – agronomic traits and reaction to major diseases and insect-pests in the NW Himalaya

Trait	CM 212	VQL 1	CM 145	VQL 2	CML170	CML 180	Vivek Maize Hybrid 9	Vivek QPM 9
Plant height (cm)	144	155	144	135	190	180	195	195
Reaction to Turcicum blight	1.5	1.3	1.5	1.5	1.5	1.8	1.0	1.0
Days to silking	53	53.5	53.2	53.7	62	60	51	52
Days to maturity	85-90	85-90	85-90	85-90	110–112	108–110	85–90	85–90
Protein content (%)	9.01	8.1	9.8	8.4	8.9	8.7	9.5	8.5
Tryptophan (%)	0.42	0.52	0.55	0.58	0.87	0.9	0.59	0.83
Kernel hardness	Hard	Hard	Hard	Hard	Hard	Hard	Hard	Hard
Grain yield (t/ha)	4.03	4.07	3.39	3.44	4.1	CML 180	5.9	5.8

Multi-location trials of the MAS-converted QPM hybrid under AICRP

The new hybrid, Vivek QPM 9 possesses all the good quality of Vivek Hybrid 9 with added advantages of 30% higher lysine and 44% more tryptophan. Better quality of protein in QPM is expected to help in reducing protein malnutrition among rural masses.

Fig 03: Field evaluation of the MAS-converted QPM inbreds and hybrid in Multi-location trials of the MAS-converted QPM hybrid under AICRP

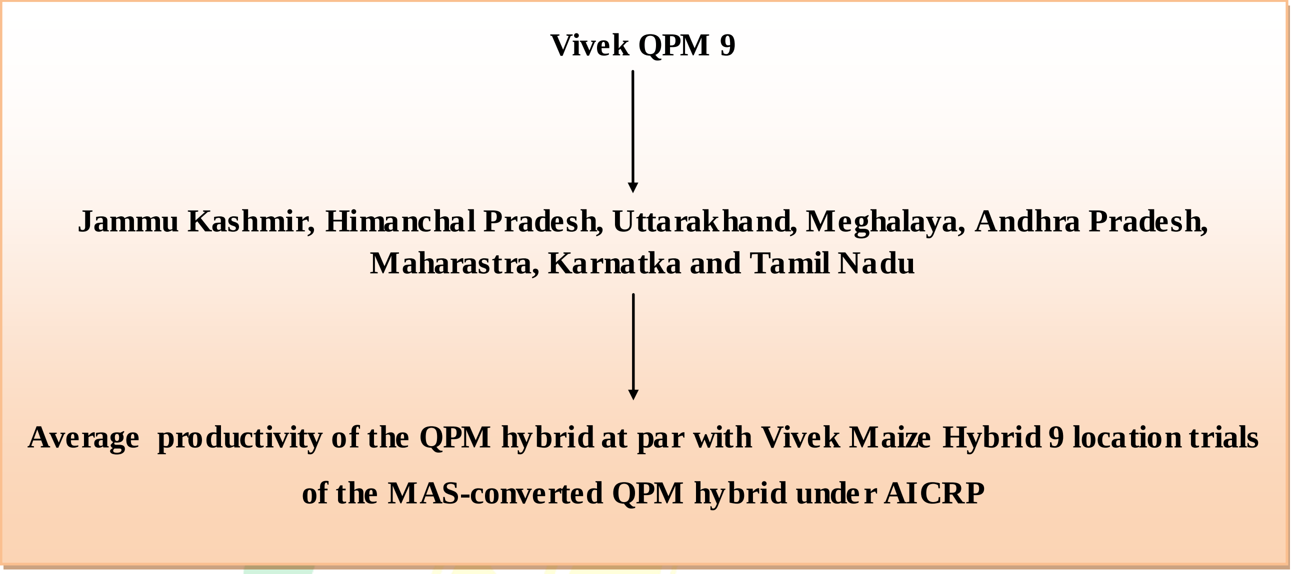

Table 04: Comparison for quality parameters of Vivek Maize Hybrid 9 and Vivek QPM 9

Hybrid	Vivek Maize Hybrid 9	Vivek QPM 9	Percent increase/decrease over Vivek Maize Hybrid 9
Protein (%)	9.5	8.5	1.0
Tryptophan (% protein)	0.59	0.83	41
Lysine	3.25	4.19	30
Leucine	14.10	12.36	-12
Histidine	2.75	3.15	23
Methionine	1.84	1.91	3.8

Recently development

- Following this approach, we have now developed another elite QPM hybrid-FQH 38
- The QPM version of Vivek Maize Hybrid 21.
- The new QPM hybrid contains more than 72% increase in tryptophan over Vivek Maize Hybrid 21
- Anand QPM 1- developed from Anand Agriculture University.

Conclusion

For a country like India, with diverse agro climatic and soil situations, we need to develop a number of QPM hybrids of different maturity groups, viz. early, medium and late (full season), so that farmers can select the right hybrids which fit in their cropping sequence. Thus, QPM can successfully replace normal maize in these areas too. However, the major constraints in adoption of the QPM hybrids in these areas are the non-availability of hybrid seeds and lack of incentives like premium price for the QPM over normal maize grains. There is also a need to create awareness among the consumers and industry for its use in food and feed.

We are developing a linkage between the seed producers, farmers and the industry to bring about the much needed synergy in development and utilization of QPM that will help in reducing protein malnutrition.

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