Genetic Improvement for Protein Content and Quality in Coarse Cereals

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INTRODUCTION

Coarse Cereals

Coarse Cereals are a group of highly variable members of family *Poaceae*, which are grown all over the world for food, feed, forage and as industrial raw material. They are functionally or agronomically related, although do not belong to the same taxonomic group.

WHAT CONSTITUTES COARSE CEREALS?

The term encompasses

- Maize, sorghum, pearl millet (major coarse grains)
- Barley, oats, rye and any of the small-seeded cereal and forage grasses (as minor millets)
- Pseudocereals and triticale
- Initially maize and sorghum were reported under coarse grains
- Now attained separate status owing to their enhanced genetic progress and increased use. (FAO Corporate Document Repository)
- Crop production statistics reported - total for the millets and sorghum

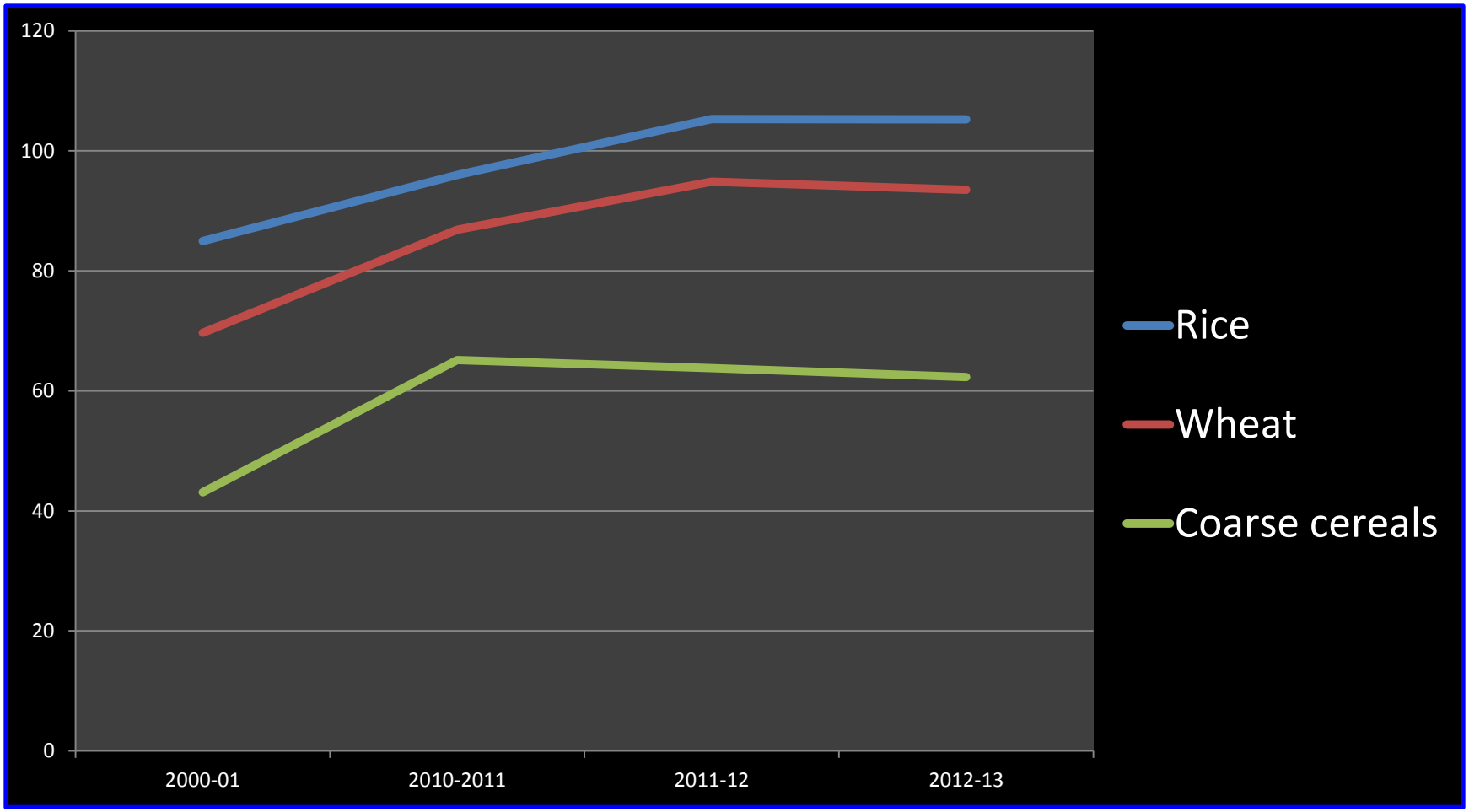
Area, production and productivity of coarse cereals (world)

Coarse cereal	Area (mha)	Production (mt)	Productivity (kg/ha)
Maize	161.77	840.31	5194.60
Sorghum	40.94	55.72	1361.20
Barley	47.59	123.54	2595.80
Oats	9.08	19.62	2161.90
Millet	34.79	31.58	907.80
Rye	5.33	12.37	2319.40
Triticale	3.94	13.35	3385.60
Pseudo cereals	8.07	11.18	1385.14
Total	311.51	1107.68	3555.84

Area production and productivity of coarse cereals in India (FAO, 2013)

Coarse cereal	Area (mha)	Production (mt)	Productivity (kg/ha)
Maize	9.50	23.29	2451.6
Sorghum	6.18	5.28	854.4
Barley	0.78	1.75	2243.6
Millet	9.20	10.91	1186.0
Total coarse grains	26.93	39.80	1478.0

Cereals: Production trends in India (Million tonnes)



Target & Achievement of production of major Food crops

India	2012-13	
Crop	Target	Achievement
Rice	104.00	105.24
Wheat	88.00	93.51
Pulses	18.24	18.34
Coarse Cereals	44.00	40.04
Food grains	254.24	257.13

Reasons for the neglect of coarse cereals

- Less remunerative in comparison to commercial crops.
- Lack of financial support in terms of subsidy for cultivation.
- No assured irrigation and no crop insurance .
- Food habit of majority of the population is rice and wheat.
- Shrinking markets.
- Lack of popularity.
- Lack of substantial research.

Importance of coarse cereals

- Highly nutritious.
- Predominantly used in our traditional cuisine.
- Provide nutritional and fodder security
- "Nutri cereals' (instead of coarse cereals)
- **Main component of food basket of the poor**
- **Grown -resource fragile regions.**

Protein contributions of cereal grains to various regions of the world.

Region	Protein intake (g)	Protein from cereals (%)
North America	105.7	18.0
Western Europe	94.8	29.0
Eastern Europe and USSR	103.3	37.0
Latin America	65.5	38.0
Africa	55.0	51.0
Near East	73.5	62.0
Far East	48.7	63.0
All developed countries	99.1	30.0
All developing countries	57.3	55.0
World	68.8	45.0

Why improve protein content in coarse cereals?

- The major portion of the protein requirement by seed proteins.
- Eight cereal -56% - energy and 50% -protein
- Developing countries- plant proteins are the main /only source of protein
- Coarse cereals -livestock feed and human food. Millions of people, particularly in the developing countries
- Millets major food - arid and semiarid regions (Africa, some parts of China, India, Korea etc.), feature in the traditional cuisine of many others
- Cereal proteins deficient -lysine and tryptophan
- Presence of antinutritional compounds, toxic compounds, enzyme inhibitors, *etc*.,
- Long term consumption of imbalanced protein diets can result in protein malnutrition

Amino acid distribution in cereal, legume and animal food sources (Young et al., 1994)

			(mg/g protein)	
Food	Lysine	Sulfur amino acids	Threonine	Tryptophan
Cereal grains	31±10	37 ±5	32 ±4	12 ±2
Legumes	64±10	25 ±3	38 ±3	12 ±4
Animal foods	**85 ±9**	38	**44**	12

Strategies used to address protein deficiency

- Food diversification
- Fortification of food with essential amino acids,
- Supplementation with good quality protein
- Minimizing the damage to nutritional value of protein during processing and storage and
- Genetic improvement of protein content and quality

However,

- Poorer sections cannot access balanced diet
- Soln: Genetically fortified with high quantity and quality of proteins.
- It would add nothing to the cost of production.

The difficulties in breeding for high protein cultivars

- Difficult to phenotype
- Lack of rapid and accurate analytical techniques for small quantities
- Negative correlation between grain protein content and yield
- Low genetic variability and requirement for accurate analysis
- The experience in maize - chalky endosperm, dull appearance and high moisture content of seeds -susceptible to storage pests and diseases with reduced yield (
- Complex inheritance pattern, and hence are difficult to work with

Seed storage proteins

- ✓ Seed - main plant part -consumed in majority of food crops
- ✓ In seeds, majority of the individual proteins are present which have either metabolic or structural roles in the seeds.
- ✓ In addition, -storage proteins-store of amino acids
- ✓ storage proteins -protein content and quality.

Seed storage proteins (Osborne, 1924)		
Protein	**Solubility**	**Major source**
Albumins	Water	Dicots
Globulins	Dilute salt	Dicots
Prolamins	Aqueous alcohol	Monocots
Glutelins	weak acid or alkali	Monocots

Major seed storage proteins in coarse cereals

Coarse cereal	Major Storage protein	Prolamin Fraction	Percent of total protein
Maize	Prolamin	Zein	50-60%
Sorghum	Prolamin	Kafarin	50-60%
Barley	Prolamin	Hordein	30-40%
Oats	Prolamin	Avenin	10-12%
Rye	Prolamin	Secalin	60%
Pearl millet	Prolamin/ Glutelin	Pennisetin	Codomi-nating
Foxtail millet	Proalmin	Setarin	~50%

Genetic variation in seed proteins of coarse cereals

- Literature suggests -total amount of protein in seeds of a given species varies significantly with cultivar.
- The distribution of different storage proteins - varies with species and cultivar.
- There is a high G x E interaction for grain protein composition of the coarse cereals
- Little point in breeding for increased seed protein content *per se*, unless some improvement in the limiting essential amino acid is achieved at the same time (Murray, 2003).

Factors responsible for variation in protein content

- Environmental factors
- Higher nitrogen application- high protein-accumulation of prolamin, increase in the proportion of the sulphur-poor fractions (Kirkaman *et al*., 1982).
- Other factors such as the density of the plant population, location, season, water and stress also contribute to variations in grain composition.
- The strong environmental influence makes it difficult to compare the protein contents of grain grown in different locations or in different years.

Protein content and quality in coarse cereals

Cereal	Crude Protein content	% total calories from protein	Protein quality (% of casein)
Maize	9.8	9.4	32.1 N 96.8 O 82.1 Q
Sorghum	8.3	11.3	32.5
Barley	11	12.5	58
Oats	9.3	16.9	59
Rye	8.7	14.7	64.8
Pearl millet	11.5	11.8	46.4

Breeding efforts for improvement of protein content

- ✓ Long term selection in maize

  19 times increase in total protein content after 61 generations of -selection (Leng, 1962).

  University of Illinois-protein -10.9 to 26.6 percent in 65 generations

- ✓ Possible by back cross breeding with HP strain
- ✓ Negative correlation protein content and yield. limited interest in high protein cultivars (Simmonds, 1995).
- ✓ Considerable success in maize, sorghum and barley, which focused mainly on enhancement of lysine content using high lysine mutants or lines.

Breeding Efforts for Improving Protein Quality in Coarse Cereals

- The quality of a protein -essential amino acid composition.
- Little or no variation; even in wild relatives
- Sorghum: Lysine content ranged from 71 to 212 mg/g of nitrogen
- The effort to improve methionine and tryptophan -maize
- Traditional breeding for protein quality improvement depended upon mutants
- The discovery of high lysine mutants opaque-2 (o_2) (Mertz *et al*., 1964) and floury-2 (Nelson *et al*., 1965)
- These mutants had altered amino acid profile in maize endosperm protein
- Increase in other amino acids such as histidine, arginine, aspartic acid and glycine
- decrease in glutamic acid, alanine and leucine
- Leucine: isoleucine ratio was improved and became better balanced

Examples of High lysine mutants/ines

Coarse cereal	Mutant gene/Line	Mutant obtained by	Improvement
Maize	*o2* , *fl2*	spontaneous	two fold lysine
	o7, *o6*, *fl3*	spontaneous	inferior to *o2*
Barley	Hiproly	spontaneous	
	Risø mutant M-1508	Induced	40% more lysine
Sorghum	IS 11167	Spontaneous	15.7% protein, 3.33% lysine
	IS 11758	Spontaneous	17.2% protein, 3.13 % lysine
	P721	Induced	60% more lysine
Pearl millet	TF 23A x D356	High P stocks	17.8% P, 2.55 % lys
(W or Y	D 118		21.7% P, 1.97 % lys
endosperm)	Bichpuri local		23.0% P, 1.98 % lys

Approaches for Genetic Engineering

(i) homologous gene manipulation - the coding sequences of native seed protein gene altered by site-directed mutagenesis (addition, substitution or deletion of nucleotides or combination the combination of there of). Such modified gene is reintroduced under its own promoter into the target plant; or

(i) Heterologous gene transfer- The gene/genes encoding higher levels of protein or desired amino acids from a different species is introduced into the plant of interest

Approaches for Genetic Engineering

Increasing proteins rich in essential amino acids in coarse cereals:

- Genes encoding lysine rich proteins
- beta-amylase (5%), protein Z (7.1%), chymotrypsin inhibitors CI-I (9.5%) and CI-2 (11.5%) and hordeothionin of barley

Reducing the synthesis of proteins poor in essential amino acids

- In maize RNAi -used to down-regulate the lysine poor zeins. upto 16-20% more lysine
- Double stranded RNA (ds RNA) - a refined approach to simultaneously down-regulate both 22 KDa and 19 KDa α-zeins resulting in increase in lysine from 2.83 to 5.62% and tryptophan from 0.69 to 1.22%

Increasing the level of free amino acids

- Small pools of free amino acids are also present (1% or less of the total)
- The transfer and expression of enzymes that are insensitive to Lys feedback inhibition
- E.g. Sorghum

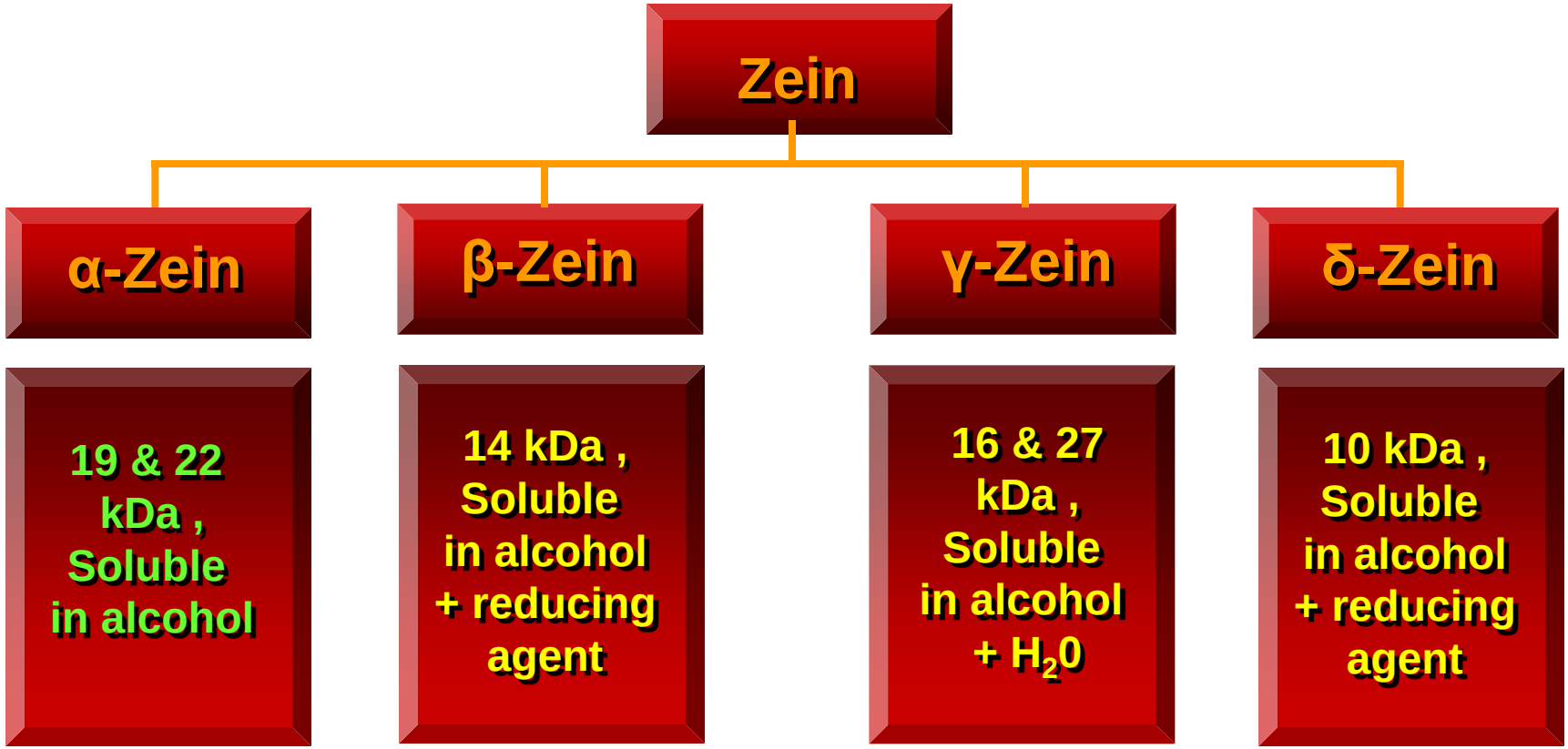
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Protein Quality in Maize

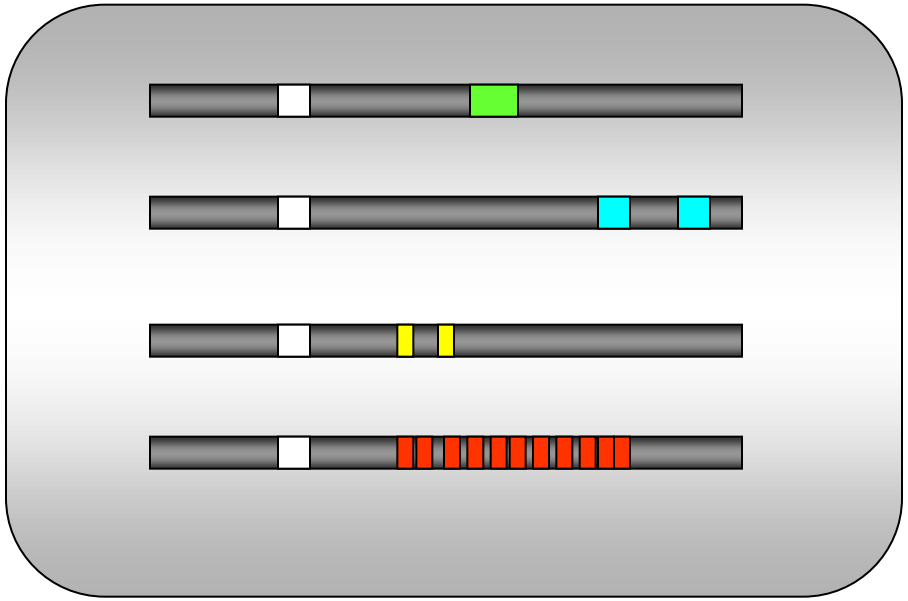
- Human including monogastric animals can not synthesize lysine and tryptophan.
- 90% of the maize grain proteins are in the endosperm.
- Endosperm proteins deficient in lysine and tryptophan.
- Germ proteins are superior in quality but a smaller proportion (12%) of total kernel reduces the availability of lysine and tryptophan.
- Therefore increasing the endosperm protein quality particularly lysine and tryptophan assumes considerable significance.

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Protein Quality in Maize



Zein coding genes in the maize genome



δ- Zein

β- Zein

γ- Zein

α- Zein

- **α-Zein is a multigene family consisting of 50-100 genes mostly in cluster**
- **β, γ and δ -Zein consists of few (1-2) genes.**

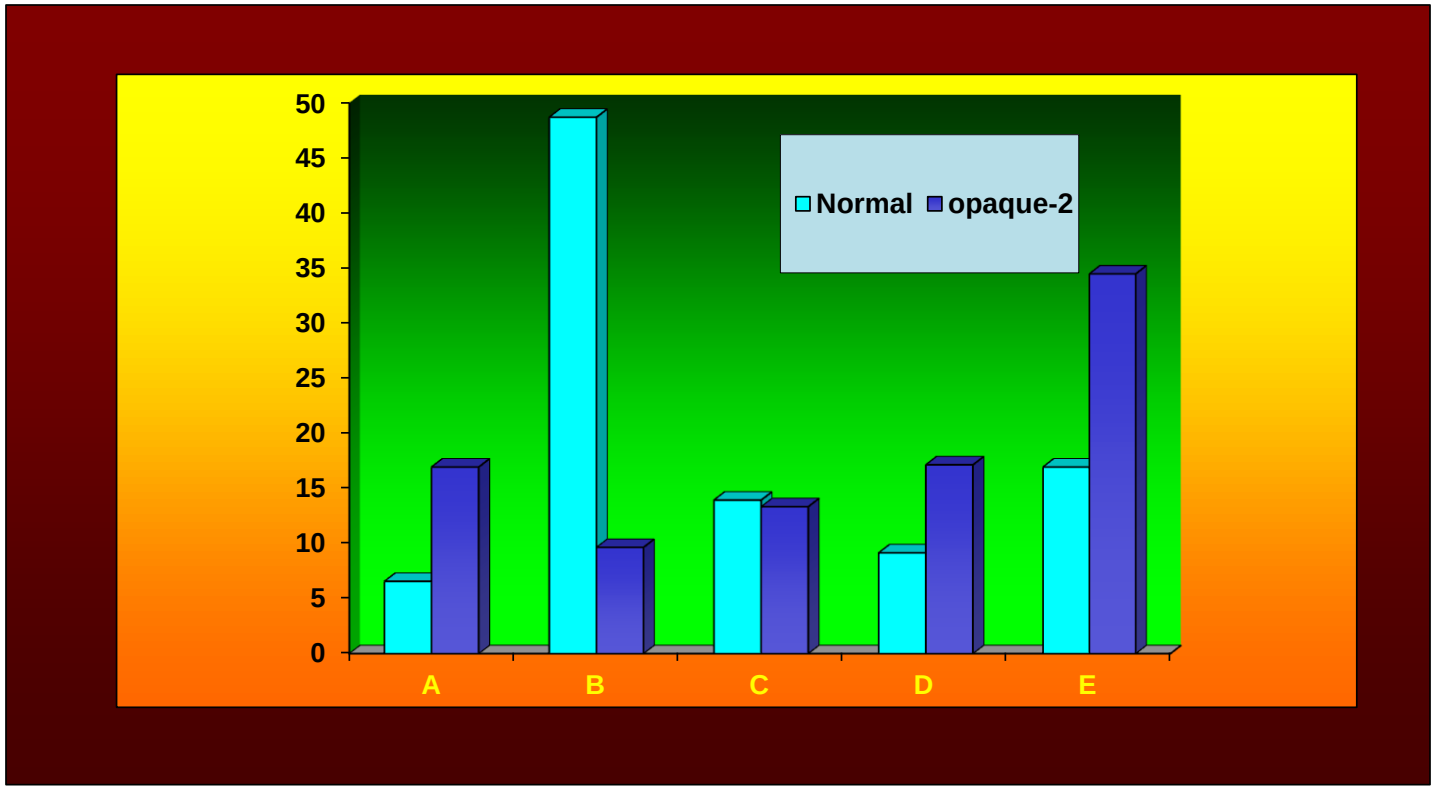
Characteristics of *opaque-2*

- Jones and Singleton in the early 1920s described the *opaque-2* mutant in maize.
- In 1964, E.T. Mertz, L.S. Bates and O.E. Nelson discovered enhanced nutritional quality of synthesizing high lysine and tryptophan.
- Recessive gene without dosage effect.
- *opaque-2* gene is located on chromosome 7
- 2-3 times increase in lysine and tryptophan
- Increase in histidine, arginine, aspartic acid and
- Glycine.
- Decrease in leucine leads to balanced leucine-isoleucine ratio and thus better digestibility.

Mechanisms of increase in lysine and tryptophan

- Decreasing the amount of zein (trace amount of lysine and tryptophan).
- Indirect increase in non-zein (higher amount of lysine and tryptophan).
- Down regulates Lysine Keto-Reductase that catalyzes lysine.

Decreasing the Amount of Zein

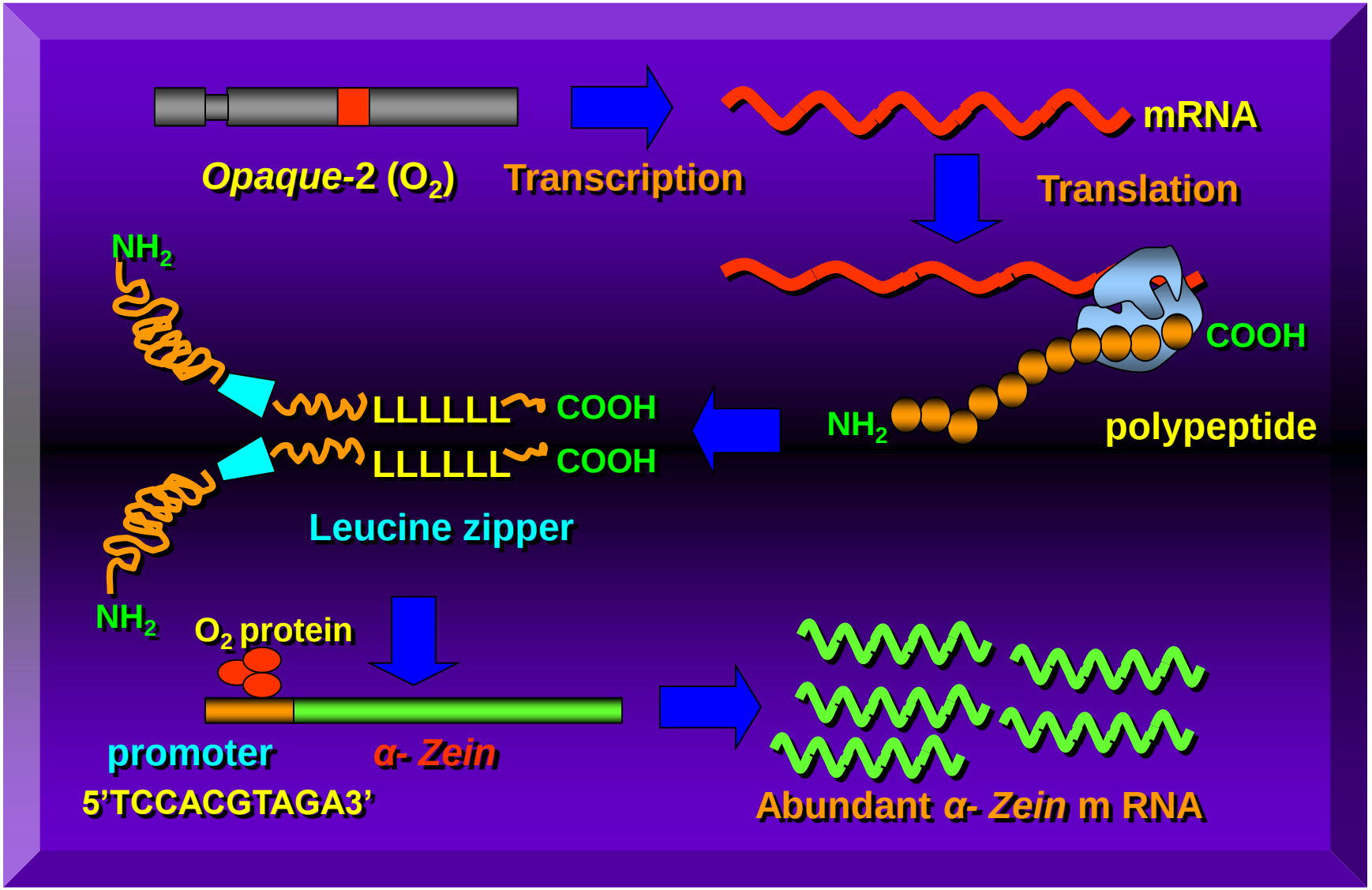


A: Albumin, Globulin and soluble N B: Zein C: Zeinlike

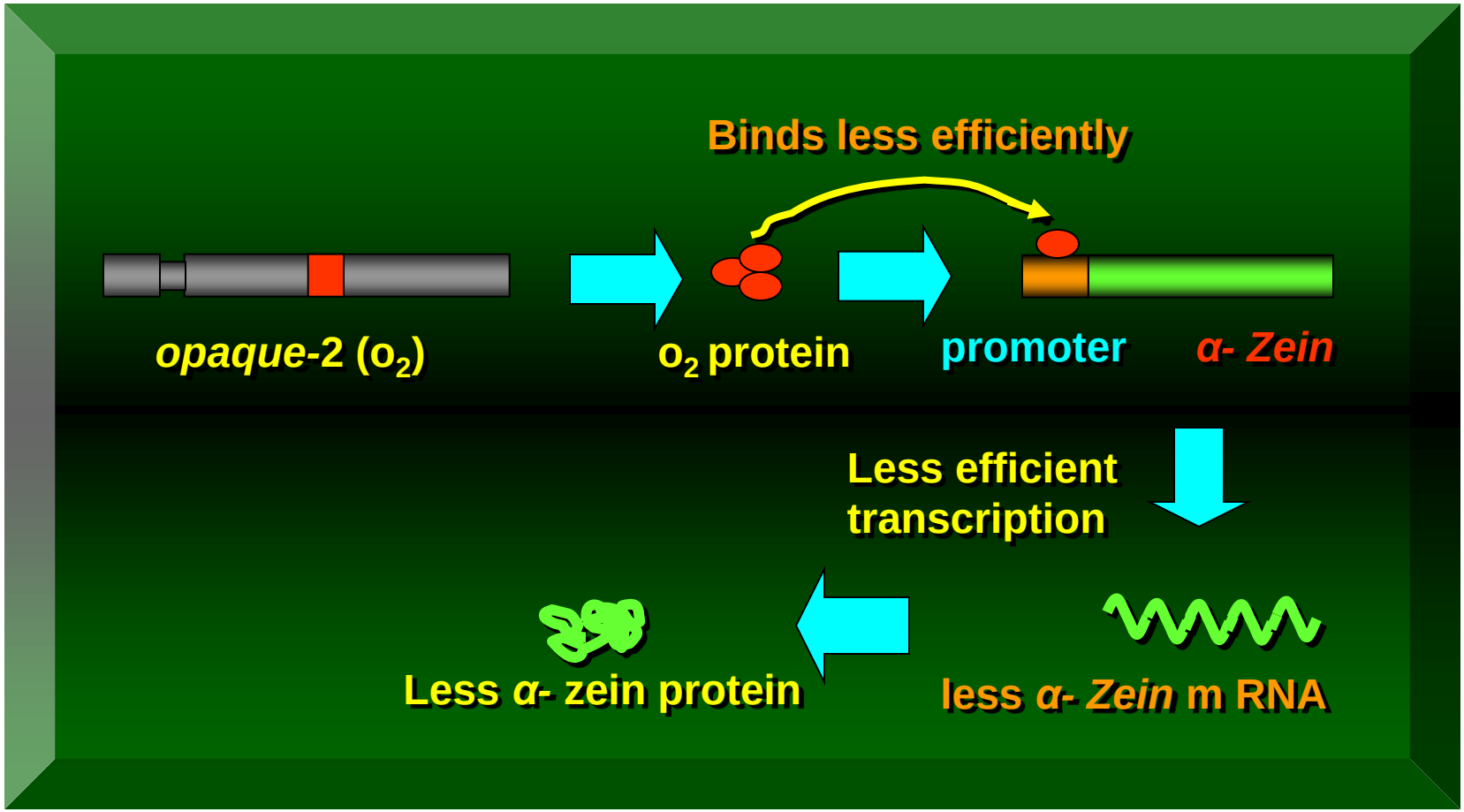
D: Glutelin like E: Glutelin

In *opaque-2*, Zein (60%) ↓ **Non-zein** ↑

Molecular Basis of *Opaque*-2 (O_2) Regulation



Mutation at Leucine Zipper Region of *opaque-2* (o_2)



Birth of Quality Protein Maize



Dr. Surinder K. Vasal

Maize Breeder, CIMMYT



Dr. Evangelina Villegas

Biochemist, CIMMYT

World Food Prize-2000

Effects of QPM on Human Nutrition

Study	Country	CM	QPM	QPM/ CM	CM	QPM	QPM/ CM
		Growth in weight (kg/month)			Growth in height (cm/month)		
1	Ethiopia	0.15	0.17	**1.15**	0.77	0.77	**0.99**
2	Ethiopia	0.11	0.14	**1.26**	0.47	0.57	**1.21**
3	Ghana	0.20	0.19	**0.95**	0.83	0.88	**1.07**
4	Ghana	0.24	0.24	**1.00**	1.03	1.23	**1.19**
5	Ghana	0.19	0.21	**1.10**	1.01	1.09	**1.08**
6	Ghana	0.35	0.38	**1.10**	1.04	1.11	**1.07**
7	India	0.21	0.26	**1.23**	0.81	0.93	**1.16**
8	Mexico	0.25	0.50	**1.97**	-	-	**-**
9	Nicaragua	0.05	0.23	**4.27**	0.35	0.58	**1.66**

Gunaratna et al., 2010

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Effects of QPM on poultry

S. No.	Diet (NM:QPM)	0-3 wks		0-6 wks	
		Body wt gain (g/bird)	Feed/ gain	Body wt gain (g/bird)	Feed/ gain
1	100:0	634b	1.454a	1464b	2.046a
2	75:25	641b	1.382b	1461b	1.991ab
3	**50:50**	**675a**	**1.341c**	**1510a**	**1.990b**
4	**25:75**	**686a**	**1.352c**	**1516a**	**1.991b**
5	**0:100**	**673a**	**1.350c**	**1522a**	**1.984b**
6	**100:0+Lys**	**676a**	**1.346c**	**1514a**	**1.973b**
	SEM	0.049	0.053	0.016	0.015

50% N + 50% QPM is better than 100% N maize feed

Panda et al., 2013

QPM Hybrids released in India

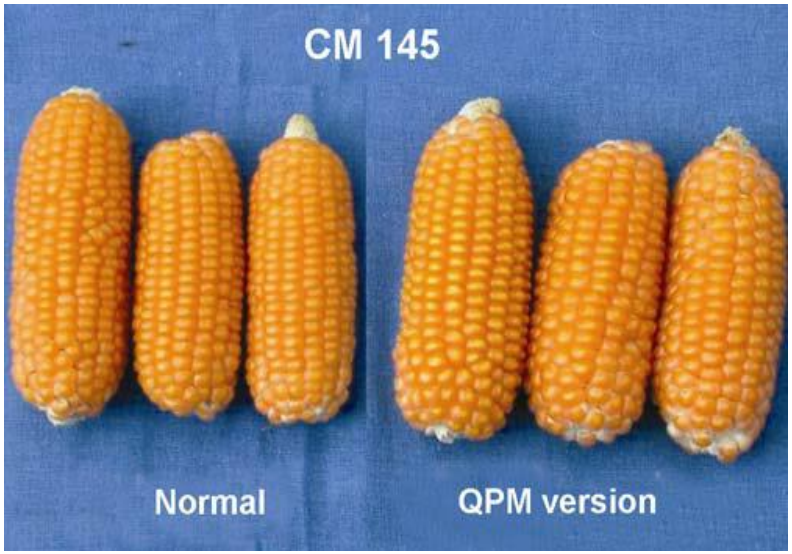
S. No.	Hybrids	Parentage	Kernel colour	Year
1.	Shaktiman-1	(CML142 × CML150) × CML186	White	2001
2.	Shaktiman-2	CML176 × CML186	White	2004
3.	Shaktiman-3	CML161 × CML163	Yellow	2006
4.	Shaktiman-4	CML161 × CML169	Yellow	2006

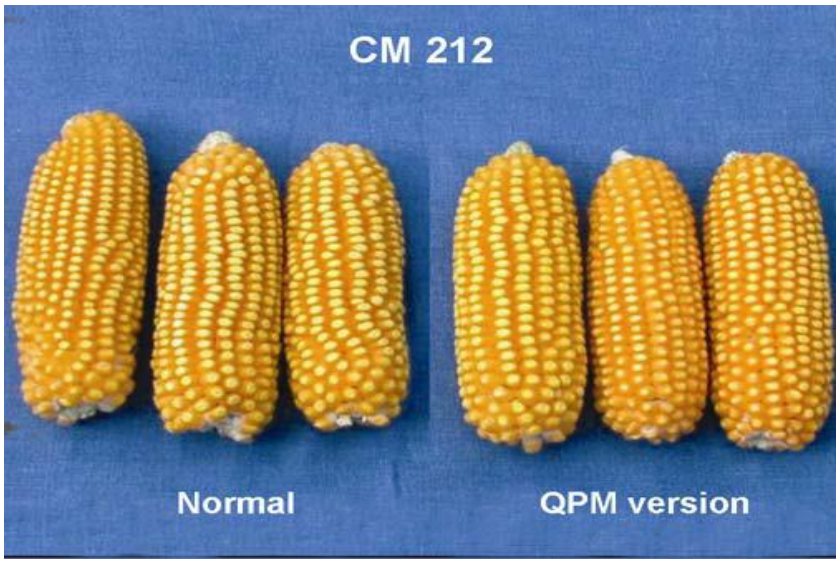
QPM Hybrids released in India

S. No.	Hybrid	Parentage	Year	Area
1.	HQPM-1	HKI193-1 × HKI163	2005	Across the country
2.	HQPM-5	HKI163 × HKI 161	2007	Across the country
3.	HQPM-7	HKI193-1 × HKI161	2008	Karnataka, AP, TN & Maharashtra
4.	HQPM-4	HKI-193-2 × HKI 161	2010	Across the country
5.	Pratap QPM Hybrid-1	DMRQPM-106 × HKI-193-1	2013	Gujarat, Rajasthan, MP, Chattishgarh
6.	Shaktiman-5	CML161 × CML165	2013	Orissa, Bihar, Jharkhand, West Bengal and Eastern UP

MAS-derived QPM inbreds

Trait	CM 212	VQL 1	CM 145	VQL 2	CML 170	CML 180
Plant height (cm)	144	155	144	135	190	180
Reaction to turcicum blight (scale 1–5)	1.5	1.3	1.5	1.5	1.5	1.8
Days to silking	53.0	53.5	53.2	53.7	62.0	60.0
Days to maturity	85–90	85–90	85–90	85–90	110–112	108–110
Protein content (%)	9.01	8.1	9.8	8.4	8.9	8.7
Tryptophan (%)	0.42 →	0.52	0.55 →	0.58	0.87	0.9
Kernel hardness	Hard	Hard	Hard	Hard	Hard	Hard
Grain yield (t/ha)	4.03	4.07	3.39	3.44	4.1	4.5



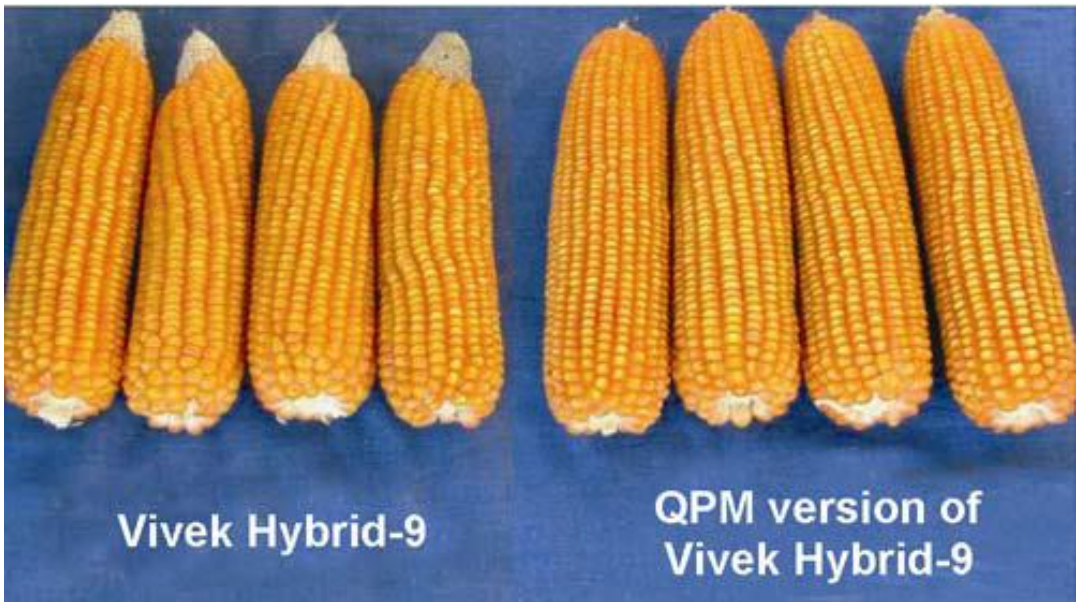


Development of Vivek QPM-9: First MAS product in maize in India

Trait	Vivek Maize Hybrid 9	Vivek QPM 9
Plant height (cm)	195	195
Reaction to turcicum blight (scale 1–5)	1.0	1.0
Days to silking	51.0	52.0
Days to maturity	85–90	85–90
Protein content (%)	9.5	8.5
Tryptophan (%)	0.59	0.83
Kernel hardness	Hard	Hard
Grain yield (t/ha)	5.9	5.8

30% lysine

40% tryptophan



- Identified for release for commercial cultivation by the AICRP on Maize in April, 2006 & 2008 (Zone I & IV); notified in Oct. 2008 from CVRC.
- Released for commercial cultivation by the State Varietal Release Committee of Uttarakhand in 2007.

Vivek QPM 21: 2nd MAS product in maize in India





Zone I: Yield Potential 70 Q/ha

MAS-derived QPM hybrids in the pipeline

S. No.	Name of the hybrids	Recipient Inbred	Donor Inbred	Institutions	Maturity
1.	Vivek QPM-9, Vivek QPM-21	VQL1, VQL2, VQL17	CML170, CML180, CML189	VPKAS, Almoa	Extra Early
2.	HM-4 , HM-8, HM-9, HM-10, HM-11	HKI323, HKI1105, HKI1128	HKI161, CML161, HKI193-1	IARI, New Delhi	Medium/ Late
3.	DHM-117	BML6, BML7	CML181	ANGRAU, Hyderabad	Medium
4.	Expt. Hybrid	HKI287, HKI1126	HKI161, HKI193-1	JNKVV, Jabalpur	Medium
5.	EHL 161708	BAJIM-26, BAJIM-27	CML169 CML193	CSK-HPKV, Palampur	Medium
6.	Expt. Hybrid	V398	CML180	ICAR-NEH Region, Barapani	Early

PAU: Buland (LM11 x LM12) and PMH-1 (LM13 x LM14) have been targeted for QPM conversion

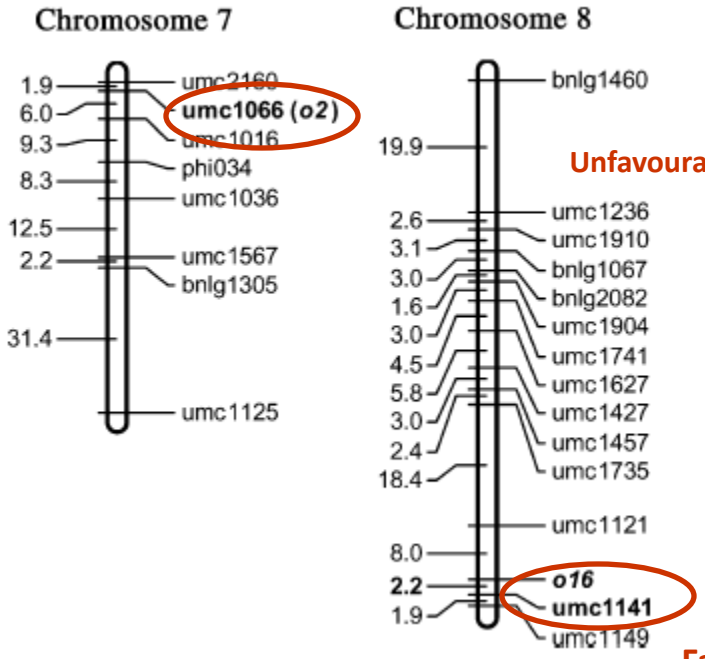
Further Enhancement of Lysine/Tryptophan by *opaque16*

Molecular Breeding (2005) 15: 257–269
DOI 10.1007/s11032-004-5947-8
© Springer 2005

Molecular genetic mapping of a high-lysine mutant gene (*opaque-16*) and the double recessive effect with *opaque-2* in maize

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o2o2/o16o16: **Increase of 40–80%, average 60%**



IARI, New Delhi

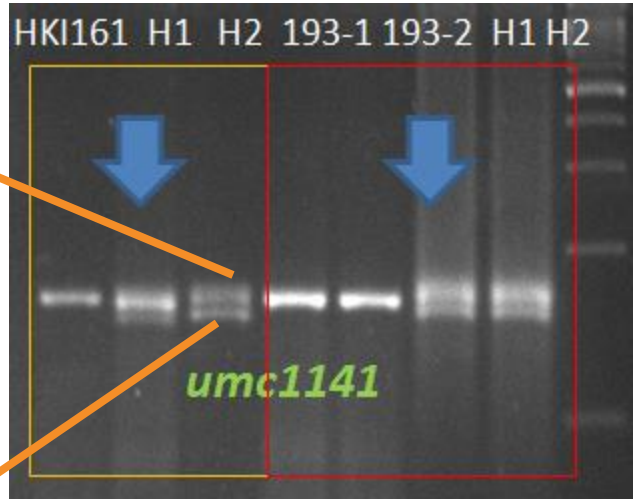
F1 = CML161 x *o16o16*
F1 = CML193 x *o16o16* ⇒ **Provided by China**

selection

CML161 ⇒ HKI161
CML193 ⇒ HKI193-1 & HKI193-2

HKI193-1 x HKI161 = HQPM-4
HKI193-2 x HKI161 = HQPM-7

Unfavourable allele



Favourable allele (*opaque16*)

Conclusions

- The nutritional quality improvement -a challenge to plant breeders.
- Number of achievements -high promise towards fighting the menace of malnutrition.
- Modern Breeding techniques-
- Enhancement of grain quality – ensure seed functionality, processing, grain yield, agronomic performance *etc*.
- Domestication & selection - loss of some other useful genes and traits; screening of germplasm for quality proteins will need to be extended to wider sources
- Not much research work in this area- opportunities and need for improvement of millets
- Success in maize and to some extent in sorghum and barley- extend to other millets
- New technologies and better understanding of the metabolism strengthen the ability to manipulate plant metabolism.
- Active collaborative efforts needed