

Quality Protein Maize (QPM)

Debra Skinner

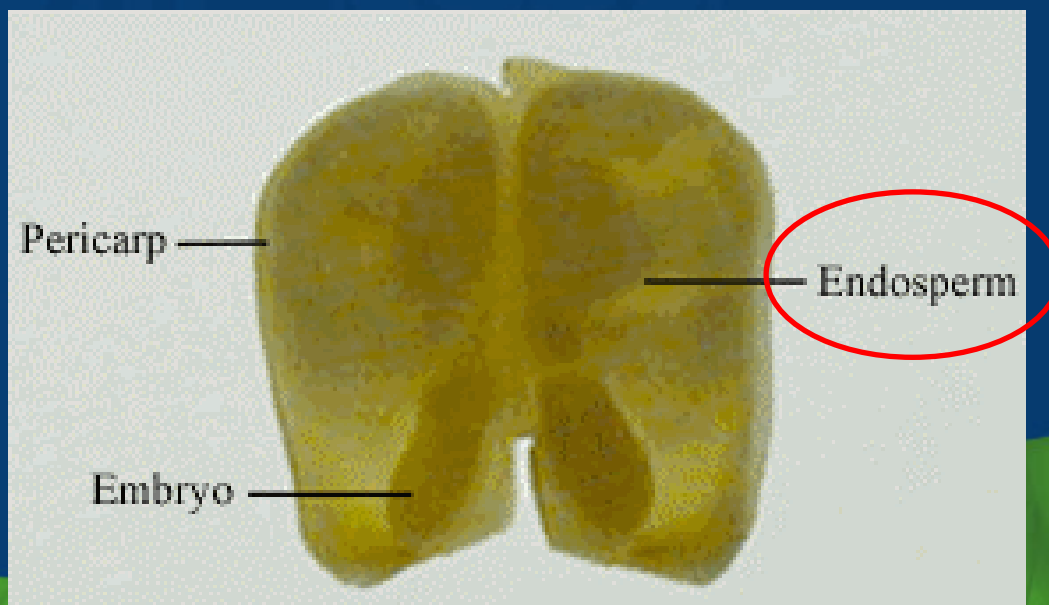
Alan Krivanek

Science behind breeding Quality Protein Maize (QPM)

- ▶ **Overview**
- ▶ **Genetics and Breeding**
- ▶ **Challenges**

Opaque-2 maize: History

- ▶ Discovered as a naturally occurring mutation in the 1920's in USA
- ▶ Lines screened for endosperm protein changes in the 1960's (Mertz, 1964).
- ▶ **lysine and tryptophan content in grain endosperm was found to be greatly increased to ~ double normal content**



Opaque-2 maize: Child Nutrition

- ▶ Desirable Effects:
 - ▶ Nearly doubles lysine and tryptophan content in grain endosperm – improved biological protein source



Protein malnourished child recovered with QPM, Colombia, 1969

	Nitrogen Balance	Quality as % of Milk
Normal Maize	0.31	39
o2o2 Maize	0.72	90
Milk	0.80	100

Bressani, et al. 1969b

Viteri et al. 1972



Opaque-2 maize: Livestock Nutrition



Guatemala 2000



EL Salvador 1999



Comparación del uso de maíz de alta calidad protéica (QPM) y normal en engorda de cerdos. Palmira, Colombia, 1970

Colombia 1974



Ghana 1994

Opaque-2 maize

- ▶ Desirable Effects:
 - ▶ Nearly doubles lysine and tryptophan content in grain endosperm
- ▶ Undesirable Pleiotropic Effects
 - ▶ Soft/chalky texture
 - ▶ Slow dry-down
 - ▶ Ear rot / storage pest susceptibility
 - ▶ Lower yields



Opaque-2 maize conversion to QPM

- ▶ Some improved grains were found by chance, and an effort to select hardness in the *opaque2* background through breeding began in the early 1970's at CIMMYT and elsewhere
- ▶ Conversion changes opaque floury endosperm to vitreous phenotype of normal maize while maintaining higher lysine and tryptophan content



Opaque-2 maize >>> QPM maize

Genetics of QPM

- ▶ The breeding of quality protein maize (QPM) involves the manipulation of **three** distinct genetic systems

Genetics of QPM

- ▶ 1st Genetic System: Recessive *o2* allele –primary locus controlling the high lysine/tryptophan content

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- ▶ *O2* gene transcription factor regulates alpha-zeins,
 - ▶ most abundant proteins in the grain endosperm
 - ▶ particularly lysine and tryptophan poor

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 - ▶ most abundant proteins in the grain endosperm
 - ▶ particularly lysine and tryptophan poor
- ▶ *O2* also regulates enzyme that degrades free lysine
- ▶ *o2o2* genotypes have
 - ▶ down-regulated zein synthesis
 - ▶ corresponding increase in non-zein proteins (which have higher levels of lysine and tryptophan)
 - ▶ more free lysine

Genetics of QPM

- ▶ The *O2* locus alleles segregate in a simple Mendelian manner.

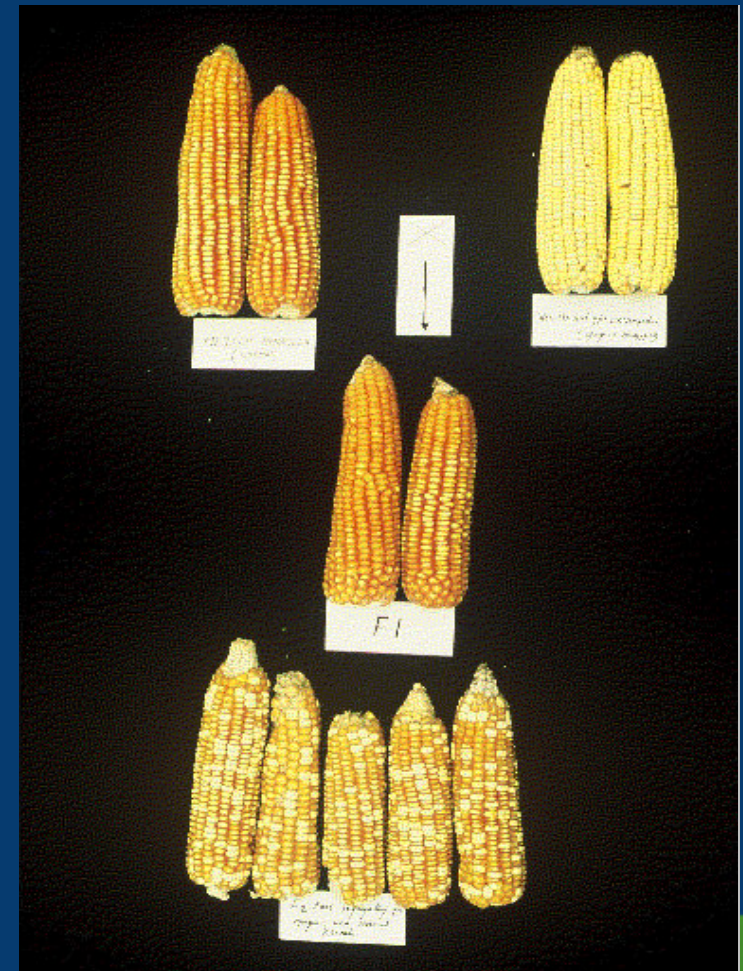
$O2O2 \times o2o2$



$O2o2$



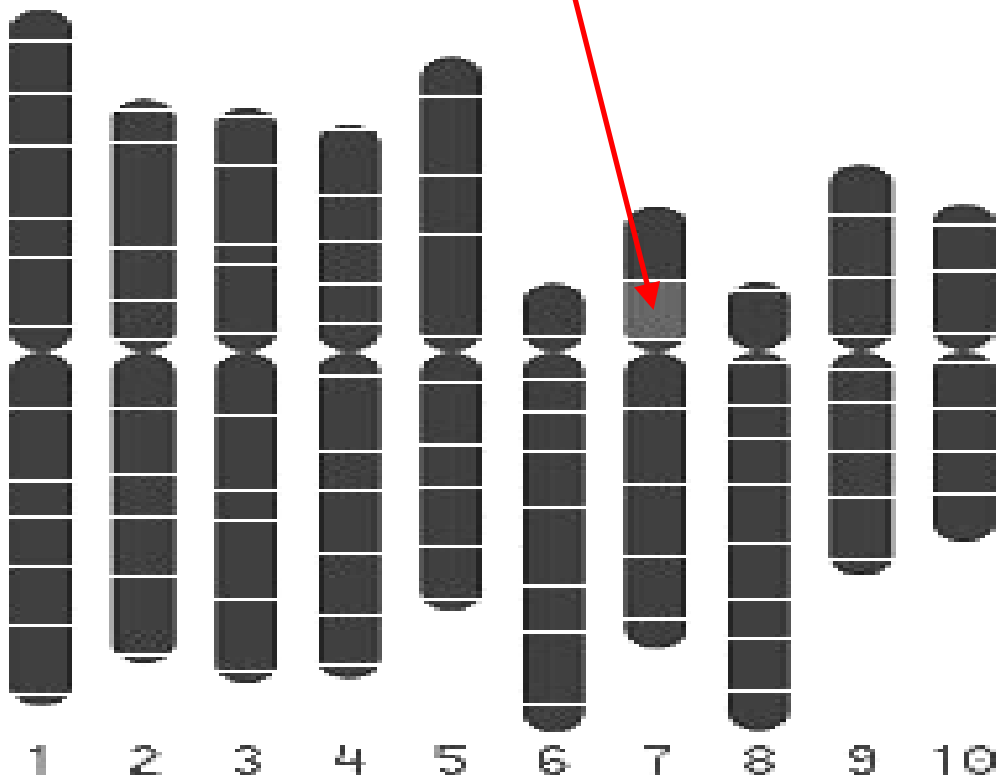
$\frac{1}{4} O2O2; \frac{1}{2} O2o2; \frac{1}{4} o2o2$



Genetics of QPM

- ▶ The *O2* locus has been localized on the maize genome and sequenced.

O2 gene
• *o2* allele, recessive



o2o2 is necessary but not sufficient

Genetics of QPM

- ▶ 2nd Genetic System: Vitreous endosperm modifier loci

Genetics of QPM

► 2nd Genetic System: Vitreous endosperm modifier loci

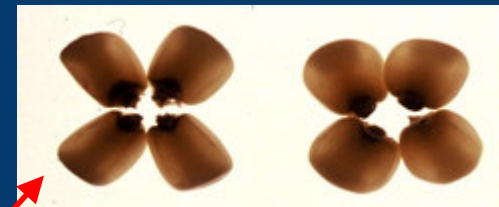
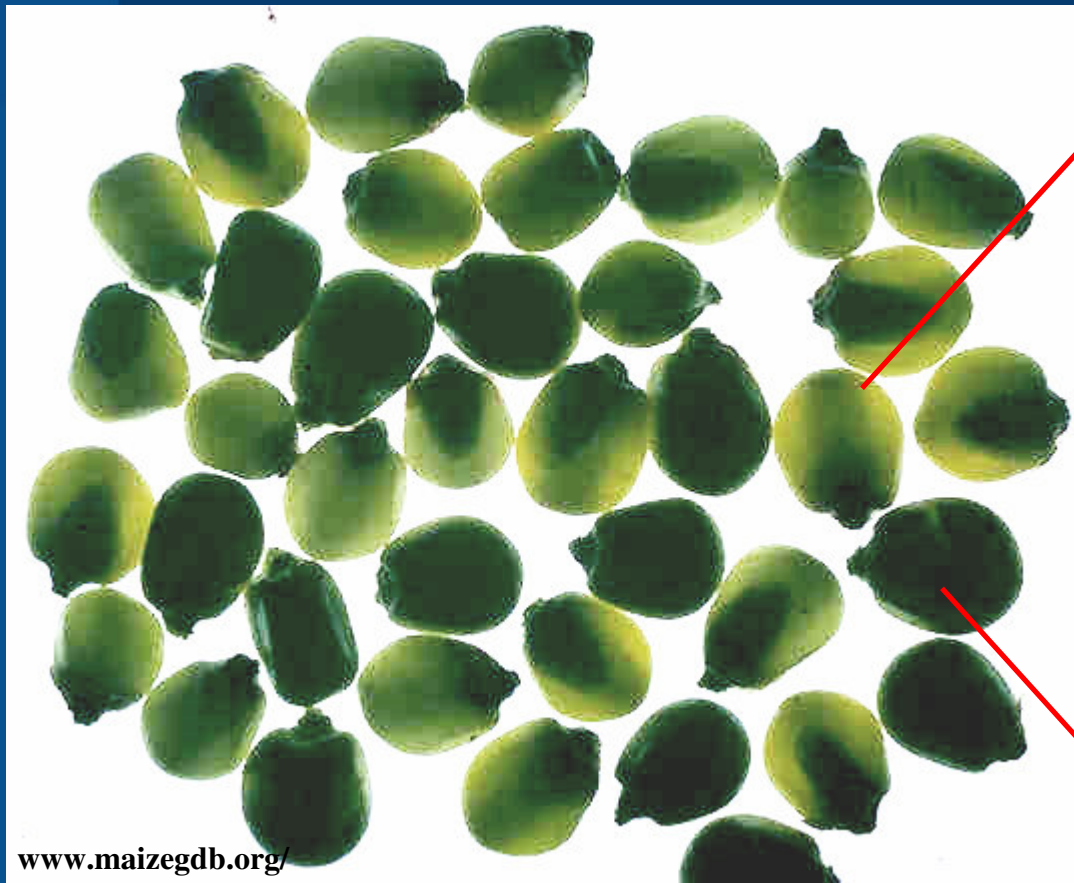


More vitreous (modified)

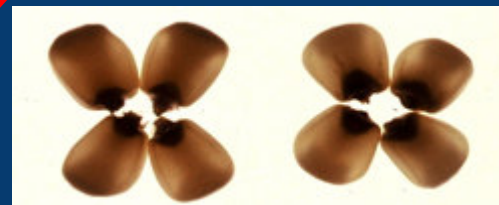
Medium modification

More opaque (not modified)

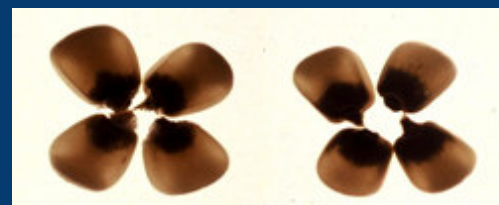
Light table vitreous endosperm scores



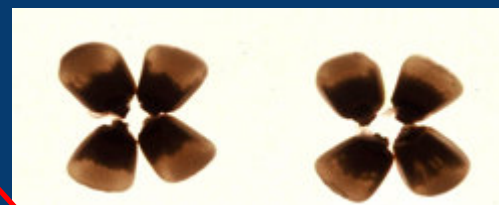
▶ 1



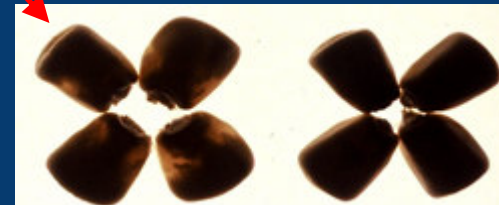
▶ 2



▶ 3



▶ 4



▶ 5

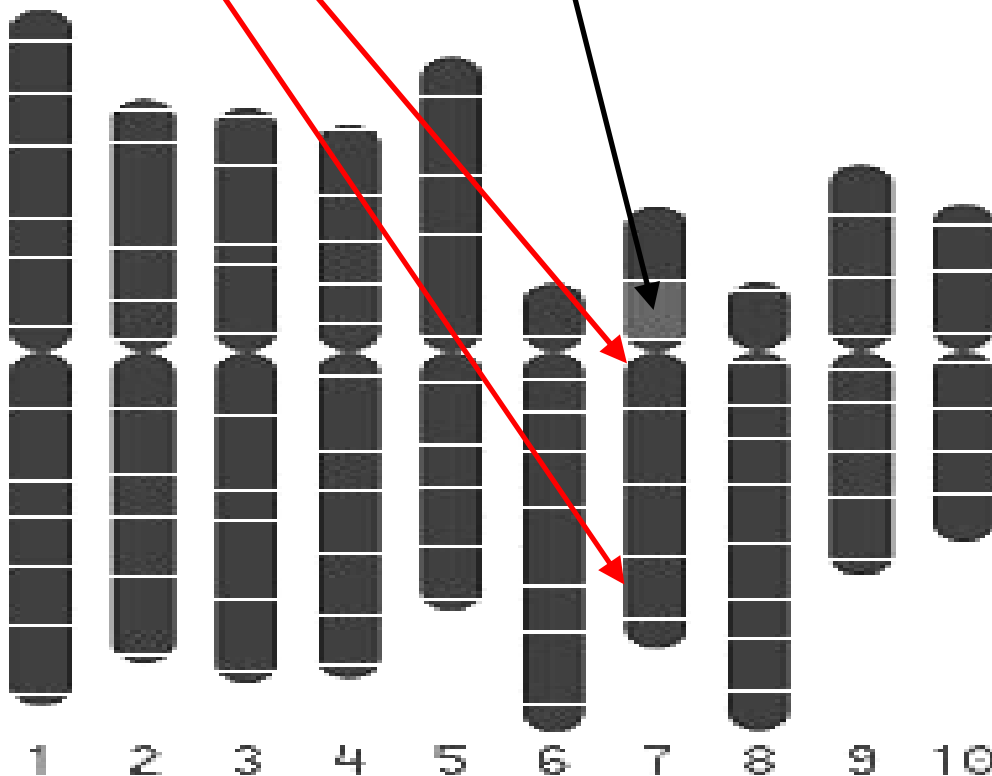
Genes/QTL effecting QPM

Modifiers- Vitreous Endosperm

- *Gzr1/o15*, ?%var, rec. (Lopes 1995)
- *vitreous*, ?%var, add. (Lopes 1995)

O2 gene

- *o2* allele, recessive



1 - *o2o2*

2 - hardness modifiers

Genetics of QPM

- ▶ 3rd Genetic System: Amino-Acid modifier loci

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 - ▶ the *o2o2* genotype does not guarantee high protein quality

	FAO Guideline Requirement for Preschool children	Normal Maize	QPM
Lysine	5.8%	1.5-2.8%	2.6-5.0%
Tryptophan	1.1%	0.2-0.5	0.5-1.1%

Moro, 1996 & CIMMYT data

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- ▶ aa acid content should be confirmed during the breeding process in order to get the most protein gain - laboratory analysis for tryptophan or lysine content is required

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- ▶ Tryptophan and lysine levels are correlated (tryptophan is cheaper to measure)

Genes/QTL effecting QPM

Modifiers- Vitreous Endosperm

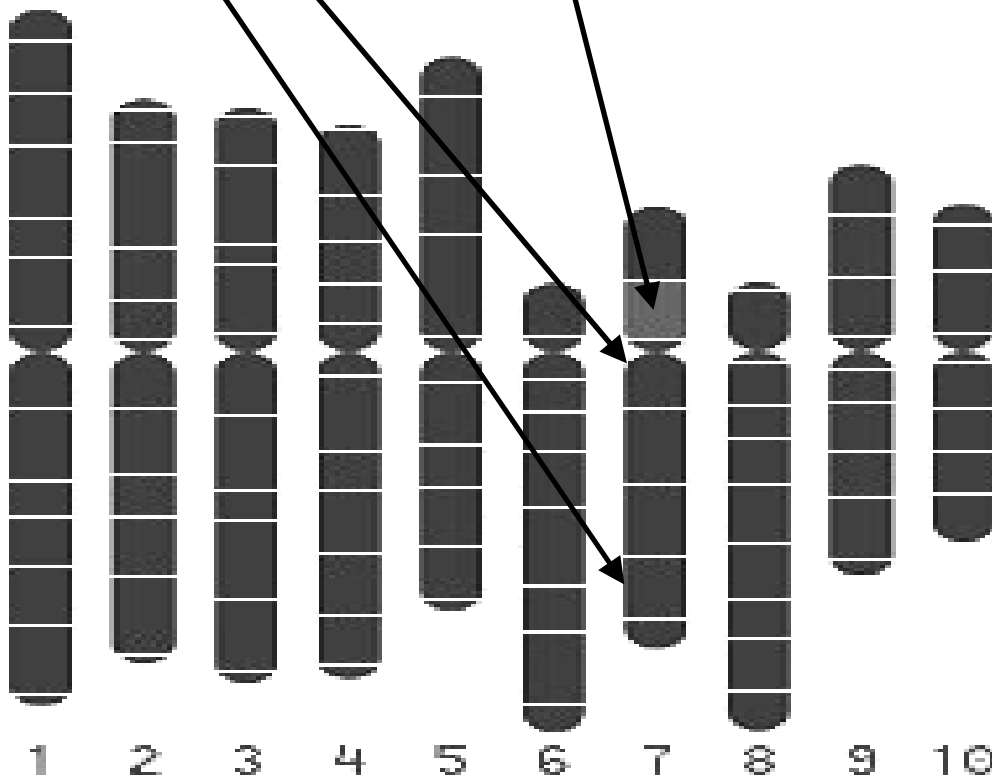
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O2 gene

- o2 allele, recessive

Modifiers- Amino Acid

- eEF1A, 7L, 14%var, add. (Wang 2001)
- eEF1A, 4S, 11%var, add. (Wang 2001)
20%var, dom. (Wu 2002)
- eEF1A, 2S, 20%var, dom. (Wu 2002)
- FAA, 1L, add/dom. (Wu 2002)
- FAA, 2S, 10%var, add/rec. (Wang 2001)
add/dom. (Wu 2002)
- FAA, 2L, 11%var, add/rec. (Wang 2001)
add/dom. (Wu 2002)
- FAA, 3S, 15%var, add/rec. (Wang 2001)
add/dom. (Wu 2002)
- FAA, 4L, add/dom. (Wu 2002)
- FAA, 5L, add/dom. (Wu 2002)
- FAA, 7L, 10%var, add/rec. (Wang 2001)
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Genes/QTL effecting QPM

Modifiers- Vitreous Endosperm

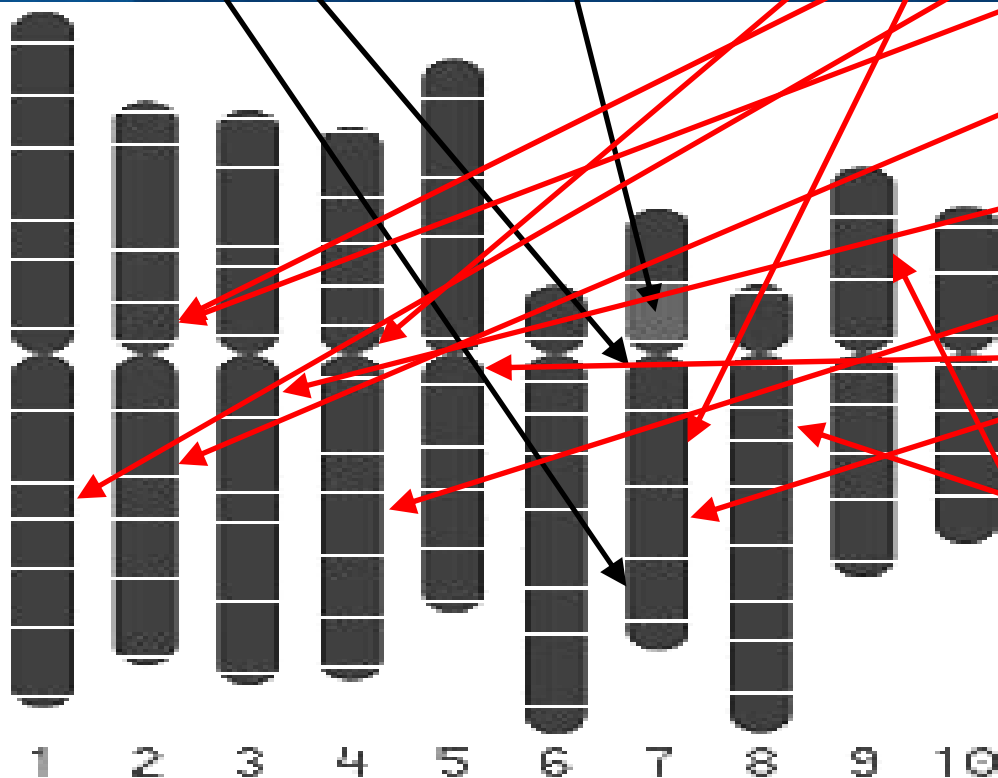
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Summary of QPM Genetic Systems

1. *o2o2*:

- ▶ allows higher lysine & tryptophan levels

2. Vitreous endosperm modifiers:

- ▶ restore endosperm hardness

3. Amino acid modifiers:

- ▶ maintain high levels of lysine and tryptophan

Monogenic trait → Polygenic trait

Breeding QPM-Backcross Conversion



Breeding QPM-Backcross Conversion

CML-264 RC3
x
CML-273 RC3
9.1 T/Ha.



CML-264 x CML-273
8.9 T/Ha



Pedigree	Yield t/ha	Endo Hard 1_5	Ear rot %	Silk days	Plant asp	Ear asp	Root Lodge %	Stalk Lodge %	Tryptophan (%Tot. Prot)
CML264 X CML273	5.27	2.2	3.8	56	2.8	2.9	6.6	2.4	0.5
CML264Q X CML273Q	5.29	2.4	6	57	2.7	2.8	3	3.2	0.91
Local Check #1	5.16	2.2	6.7	54	3.8	3.1	20.9	18.4	0.5
LSD 5%	0.65	0.5	4.5	0.8	0.5	0.3	10.4	8.9	

Breeding QPM

- ▶ Normal elite X QPM donor – Backcross Conversion

Breeding QPM

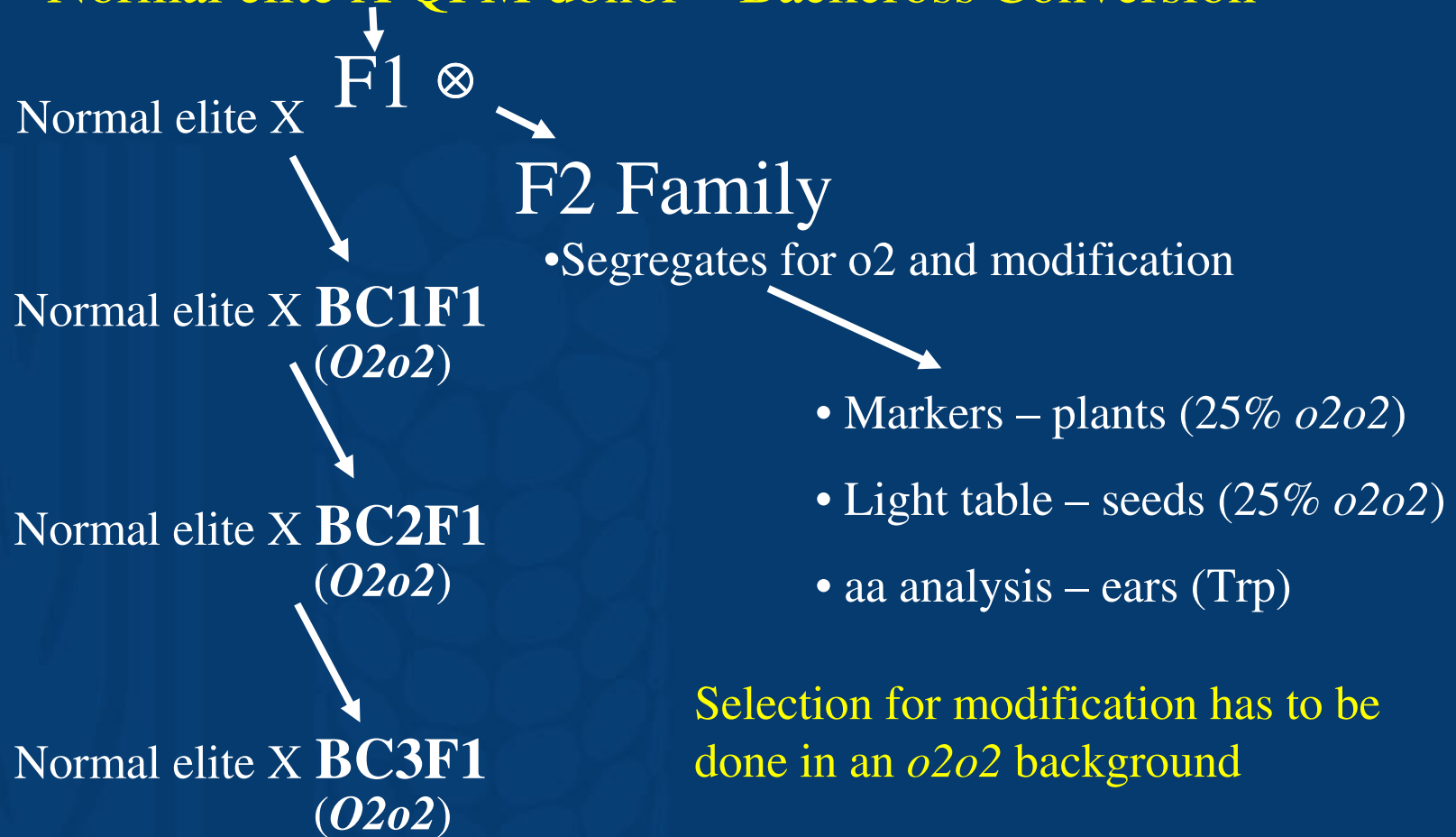
- ▶ Normal elite X QPM donor – Backcross Conversion



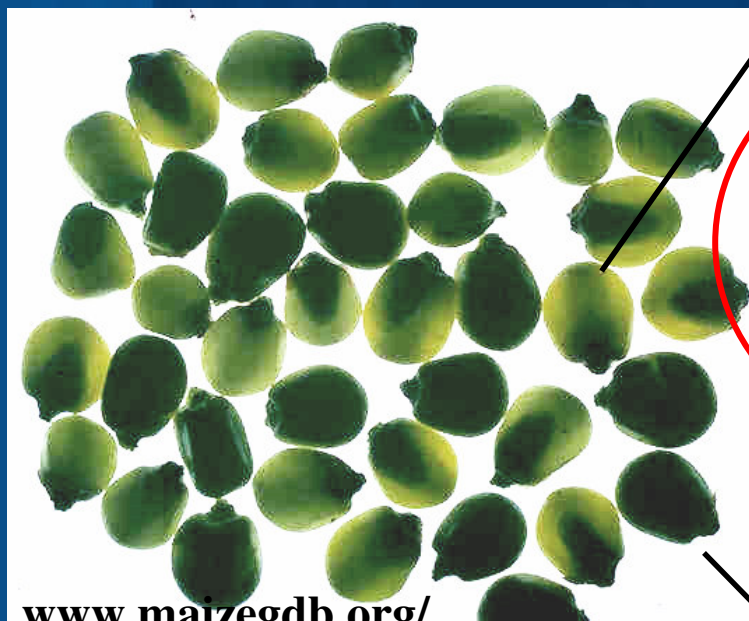
No *o2o2* during breeding, therefore no chance to select for modification

Breeding QPM

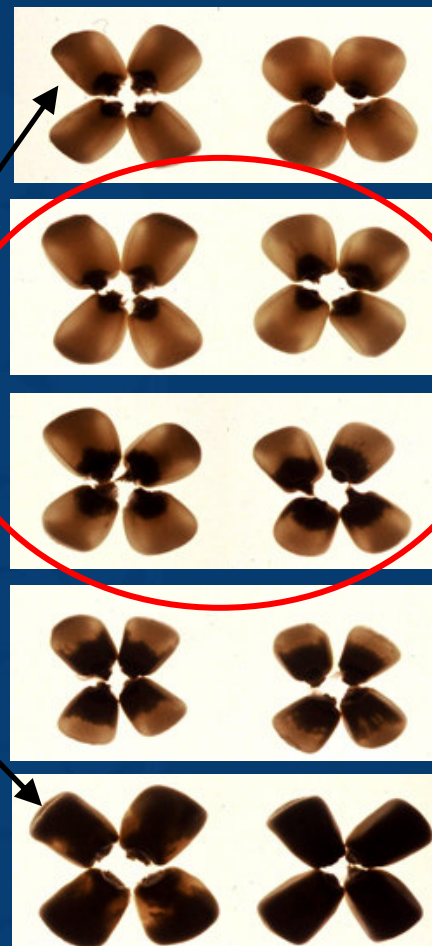
▶ Normal elite X QPM donor – Backcross Conversion



Light table vitreous endosperm scores



www.maizegdb.org/



▶ 1: *O2/O2*, *O2/o2* or fully modified *o2/o2*

▶ 2 } **More modified** ▲

▶ 3 } *o2o2*

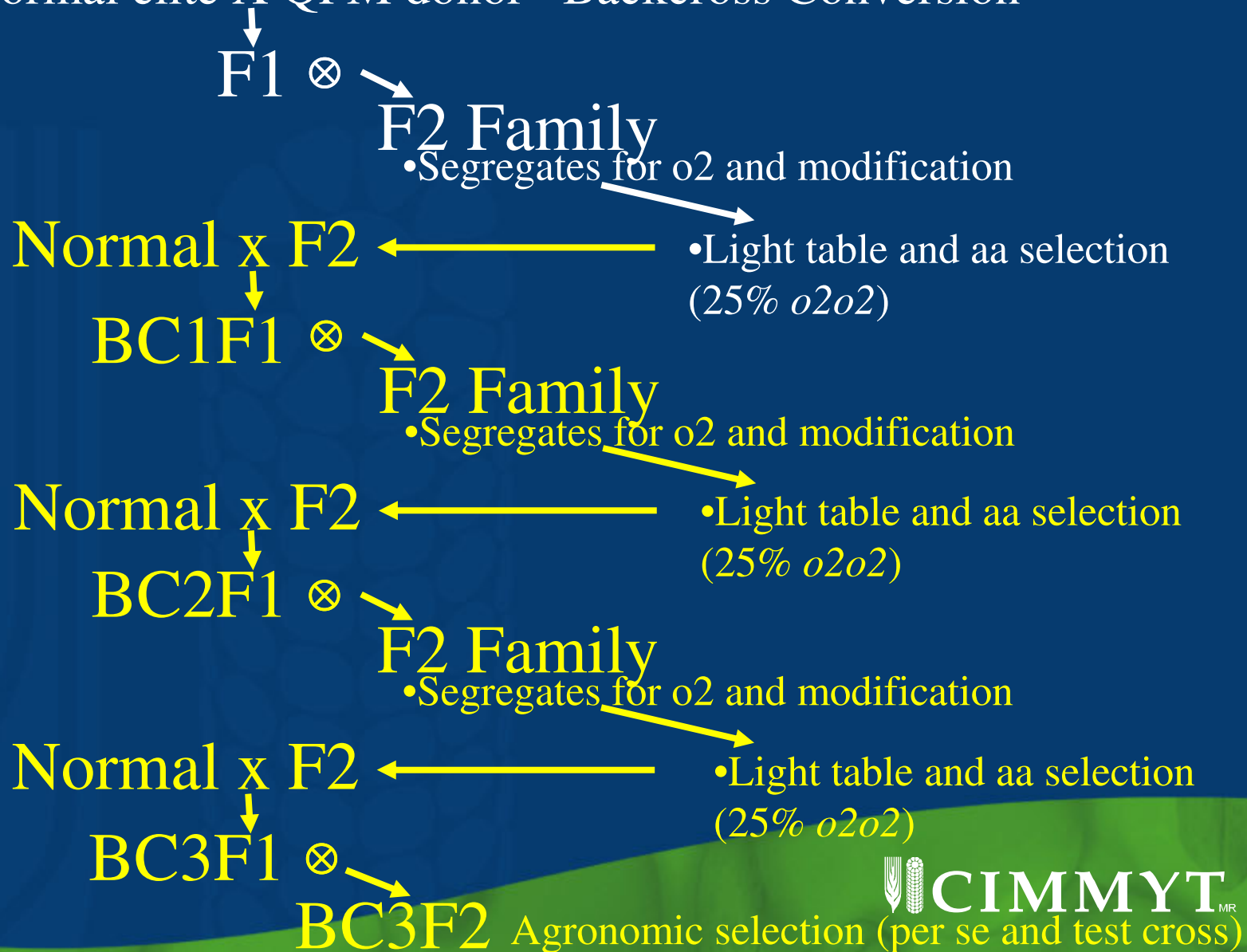
▶ 4 } **Less modified** ▼

▶ 5 } **Less modified** ▼

Breeding QPM

7 seasons to obtain BC3 seed relative to the typical 4 seasons.

- ▶ Normal elite X QPM donor – Backcross Conversion



Challenges to MAS for QPM Breeding

- ▶ Backcrossing in a recessive gene = ideal for MAS?
 - ▶ *O2* gene-based SSR and SNP markers
- ▶ But... with the modifiers, QPM is a polygenic trait!
 - ▶ No useful markers for modifiers
 - ▶ Still need F2 generation (*o2o2*) to select for modifiers
- ▶ How can the use of markers benefit QPM breeding?

Challenges to MAS for QPM Breeding

- ▶ Current procedure – no MAS
- ▶ The light table is used to sort the seeds, selecting only seeds that are score 2 and 3, hoping to ensure *o2o2* + good modification.
- ▶ These seeds are planted and the ears analysed for aa content. Only those plants with high aa content are advanced.
- ▶ Tryptophan analysis is costly (\$4.70/sample) and requires quality reagents for reliable results.

Challenges to MAS for QPM Breeding

- ▶ Markers vs. light table for *o2o2* selection on F2 seeds?
 - ▶ Have to use light table to select for hardness anyway
 - ▶ But, light table is not accurate for determining *o2o2* with error rates from 4% - 17% in two separate experiments
 - ▶ More important, error rate in category 2, 3 is higher – up to 60% error, which means up to 60% wasted plantings!
 - ▶ Also \$\$\$ wasted on tryptophan analysis of *O2/--* plants
 - ▶ **Combination of light table with marker genotyping on selected seeds?**

Targeted MAS for QPM Breeding

1. Light table used to select category 2, 3 seeds
2. Seed based genotyping to confirm *o2o2* only on these seeds (less than 20% of total number)
3. Plant only the selected seeds
 - ▶ Saves on number of plants allowing larger population
 - ▶ Saves on tryptophan analysis
 - ▶ Added cost of genotyping is relatively small due to targeted use of genotyping
 - ▶ True benefit must be determined by increases in breeding gain.
4. Late in the breeding process, could also do genotyping of category 1 seeds to find fully modified grains

Next steps for QPM (molecular) breeding at CIMMYT

- ▶ Establish efficiency of seed genotyping method for QPM
- ▶ Marker panel to pyramid *o2* alleles with Maize Streak Virus resistance alleles, Grey Leaf Spot resistance alleles and ~5 provitamin A content genes.
- ▶ Map and find markers for endosperm modifiers
 - ▶ Normal x QPM strategy: genotype the F2 to find the fully modified *o2o2* grains and compare with most opaque grains with BSA mapping
- ▶ Establish improved procedures for tryptophan analysis using glyoxylic acid (Eric Nurit & Natalia Palacios)
- ▶ Near infrared spectroscopy (NIR) for grain protein analysis
- ▶ Transgenic strategies

Challenges to QPM Deployment

- ▶ QPM cannot be differentiated from non-QPM in the field or market.
 - ▶ QPM will largely remain a hidden benefit and can be harnessed only if a critical mass of farmers grow and sell QPM in a community.
- ▶ Human nutrition: While the older studies are encouraging more evidence is being asked for by the larger public.
- ▶ Contamination leading to loss of protein quality in the field (due to the recessive *o2* allele).
 - ▶ Requires special care/training for QPM seed production.
- ▶ Some reports of reduced vitreous endosperm modification under drought stress. Needs further investigation.
- ▶ Fears: QPM is transgenic

Thanks to...

- ▶ Alan Krivanek
- ▶ Hugo Cordova
- ▶ Prior QPM breeders at CIMMYT
- ▶ Dr. Manilal William