



Simulation Approaches in Plant Breeding

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Outlines for presentation

Conventional quantitative genetics Plant breeding and quantitative genetics >QTL mapping Background of breeding simulation QuLine tool for genetics and breeding >QuLine applications Demonstration and Hands-on

Background on breeding simulation

Toluca (1000 crosses made from 200 parents) B← About 30% of Cd. Obregon (Each selected F1 harvested in bulk) the crosses are (30-80 plants harvested individually for MODPED) Toluca F2 (30-80 plants harvested in bulk for SELBLK) discarded in F1 Cd. Obregon (Bulk of 10 spikes for MODPED) F3 (Bulk of 30 spikes for SELBLK) About 1 to 3% of PLANT BREEDING crosses will derive Toluca (Bulk of 10 spikes for MODPED) IS A COMPLEX (Bulk of 30 spikes for SELBLK) lines that can be used PROCESS WITH as cultivars Cd. Obregon (Bulk of 10 spikes for MODPED) F5 MANY DECISIONS (Bulk of 30 spikes for SELBLK) (10 plants harvested individually for MODPED) Toluca F6 (40 plants harvested individually for SELBLK) About 40% of Cd. Obregon (Bulk of whole plot) F7 crosses retained Toluca and El Batan (Bulk of whole plot) F8 field tests (Reserve seed) after F7 F8 small plot evaluation F8 yield trial Cd. Obregon (Bulk of whole plot) About 20% of F9 field tests Toluca and El Batan (Bulk of whole plot) (Reserve seed) crosses retained F9 yield trial Cd. Obregon (Bulk of whole plot) F9 small plot evaluation after two rounds F10 leaf rust screening Toluca and El Batan (Bulk of whole plot) F10 stripe rust screening of yield trials International screening nursery and yield trial

Why do we need tools in breeding?

- To improve the efficiency of traditional phenotypic selection through exploring various options
- To avoid the simplified assumptions made in classical quantitative genetic theory
- To better use the large amount of gene information available from
 - Genomics research
 - January 1992: 59,317 datasets, 77,805,556 bp
 - March 2005: 43,118,204 datasets, 47,009,081,750 bp
 - QTL mapping (CAB, April 2005)
 - 3497 publications on QTL mapping
 - 1581 publications in plants

Why do we need tools in breeding?

To build a bridge between the biological data and breeders' requirements

To combine all these sources of data into "knowledge" that breeders can use in their breeding programs

Questions that can be studied by QuLine: A genetic and breeding simulation tool

- 1. Comparison of breeding efficiencies from different selection strategies and their modifications. Which breeding method should be adopted?
- 2. Balance between the number of crosses and population size of segregating generations. What shall the breeder do if the available resources increase or reduce?
- 3. Evaluation of marker-assisted selection (MAS). When and how should MAS be used?
- 4. Comparative value of single, top, back, and double crosses in breeding?

Questions that can be studied by QuLine (mainly for inbred line development)

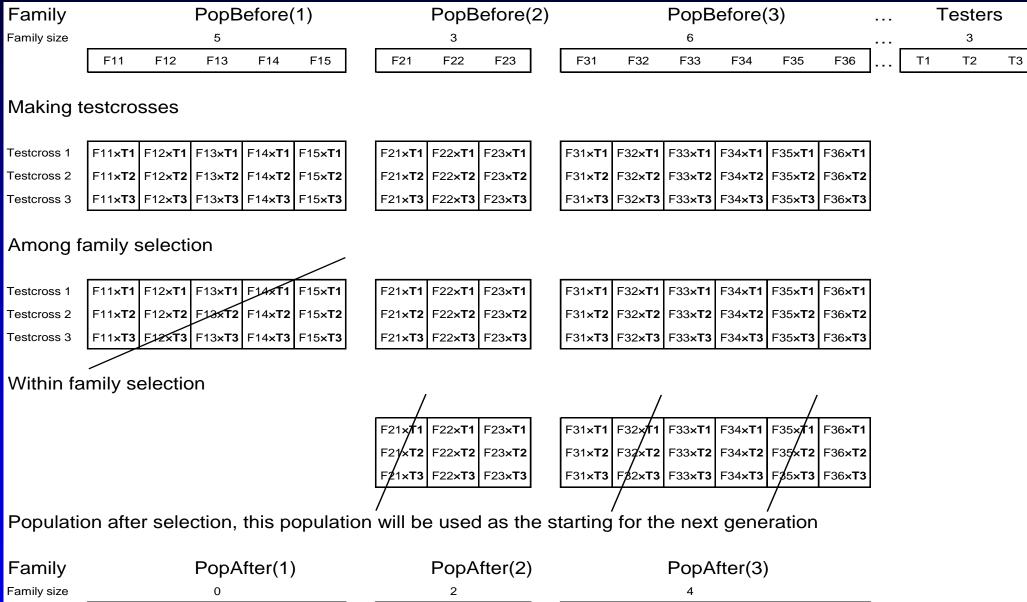
- 5. The correlation between parents and their offspring. Can F1 or F2 hybrids predict the performance of their advanced lines? For early generation selection, how early is too early?
- 6. When to use DH (doubled haploid)?
 - Early generation: many individuals need to be tested
 - Advanced line stage: lines will be good for other traits, but the desired genotype may be lost during selection due to population size, trait associations and genetic drift

QuLine tool for genetics and breeding

Available breeding simulation tools

 \geq QuLine, a computer software that simulates breeding programs for developing inbred lines QuHybrid, a computer software that simulates breeding programs for developing hybrids \geq QUMARS, a computer software that simulates marker-assisted recurrent selection and genome-wide selection

Test cross implemented in QuHybrid



F22

F23

F31

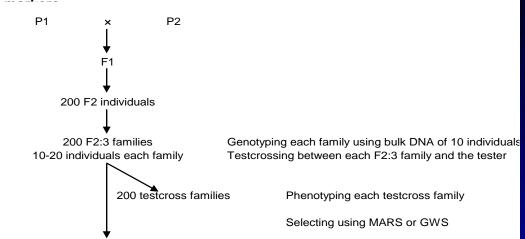
F33

F34

F36

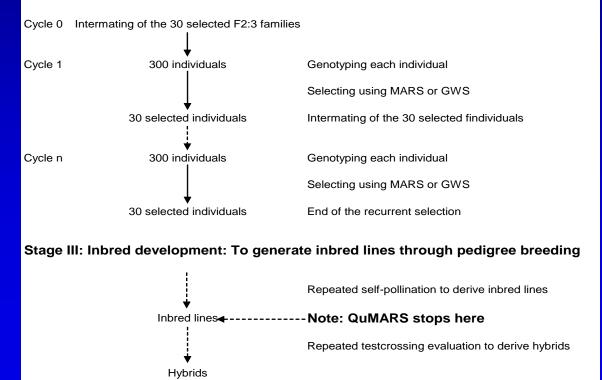
The flowchart of QuMARS

Stage I: Testcrossing: Starting from a single cross, To generate the initial population for recurrent selection; To build the prediction equation of breeding



30 selected F2:3 families based on testcross performance

Stage II: Recurrent selection: To intermate the selected families (S0); To grow and genotype randomly mated progenies; To select individuals based on MARS or GWS



What can QuLine do?

- Comparison of genetic gains from different selection methods
 - Change in population mean
 - Change in gene frequency
 - Change in Hamming distance (distance of a selected genotype to the target genotype)
- Comparison of cross performance
 - Selection history
 - Rogers' genetic distance
 - Number of lines retained from each cross
- Comparison of cost efficiency
 - Number of families
 - Individual plants per generation
- Validation of theories

In genetics (implemented by the QU-GENE engine)

- Most genetic phenomena, if not all, can be defined in the QU-GENE engine input file (QUG).
- > Among them are:
 - Multiple alleles (e.g. Glutenin genes in wheat)
 - Linkage (between gene and marker, between genes, between markers)
 - Additive, dominance and epistasis
 - Pleiotropy (one gene effects multiple traits)
 - Genotype by environment interaction
 - Molecular markers (dominant, or co-dominant)

In breeding (implemented by the QuLine module)

Most, if not all, breeding methods for selfpollinated crops, can be defined and then simulated in QuLine.

>Among them are:

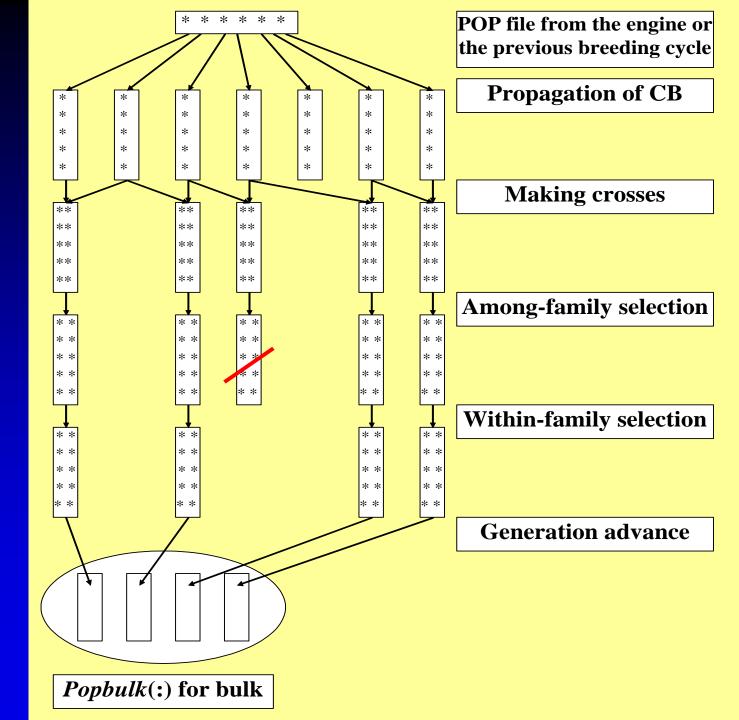
- Pedigree system (including SSD)
- Bulk-population
- Doubled haploid
- Marker-assisted selection (include marker-based selection)
- Recurrent selection within one population
- Many modifications and combinations

How does QuLine work?

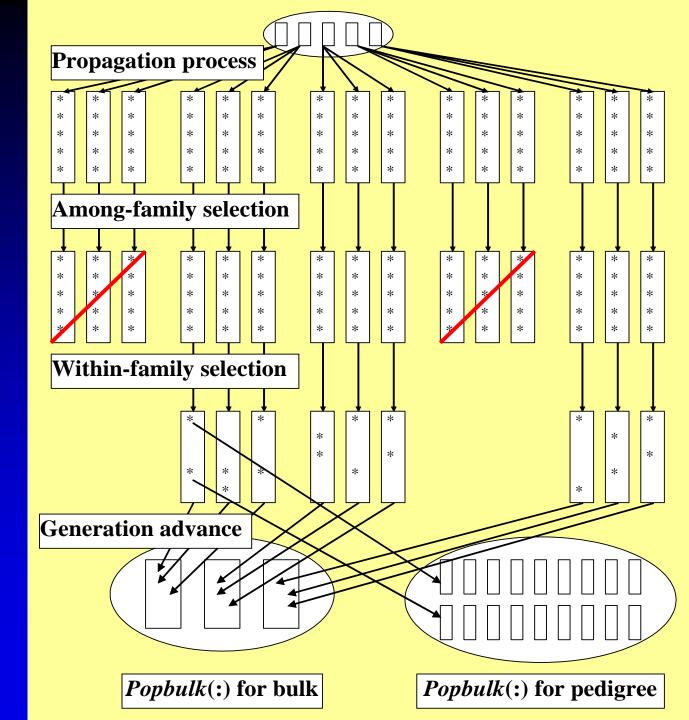
>Two input files are needed

- QUG file containing the necessary information for a genotype and environment (GE) system and initial population(s) of genotypes. It is the input for the QU-GENE engine. Two kinds of output files will be generated from the engine.
 - GES file for defining a GE system (= input for QuLine)
 - POP file for defining the initial population (= input for QuLine)
- QMP file containing the necessary information for the breeding strategies to be simulated (e.g. pedigree, bulk, SSD, DH, etc.) (= input for QuLine)

General procedure for crossing and selection in F1



General procedure for propagation and selection in F2 and onwards



Define the QMP file for the selected bulk selection method: an example

Define the breeding method in a way that the computer can understand

General simulation parameters

- Number of runs: any integer
- Number of breeding cycles: any integer
- Number of crosses in F1 generation: any integer

Indicator for crossing block update, 0 or 1.

- O: All individuals after a breeding cycle are used as the parents for the next cycle;
- 1: The best individuals in the final selected population and the initial crossing block are selected as the parents for next cycle

General simulation parameters

- Indicator for outputting GE system details, 0 for no output, and 1 for output
- Indicator for outputting population details, 0 for no output, and 1 for output
- Indicator for outputting selection history, 0 for no output, and 1 for output
- Indicator for outputting Rogers distance for each cross and the lines retained from each cross, 0 for no output, and 1 for output
- Indicator for outputting correlation coefficient, 0 for no output, and 1 for output

The number of models in the GE system and the number of runs for breeding strategy

Four nested loops in QuLine

Loop for all models

All random effects in the GE system will be assigned values Determine the parents for all crosses for the first cycle

Loop for all runs

Loop for all strategies

»Loop for all cycles

All strategies start from the same point (same crossing block and same crosses), so that they can be properly compared. Parameters to describe a set of breeding strategies

Number of strategies

For each strategy

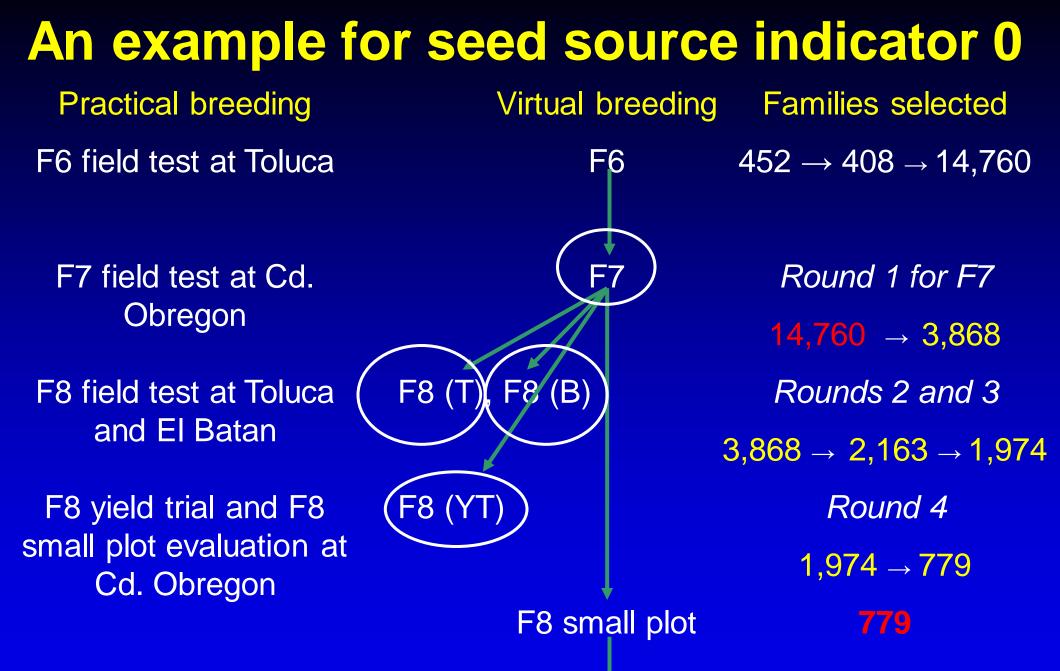
- Strategy name: any character
- Number of generations in the strategy: any integer more than 0
- Definition for each generation

Definition of a generation

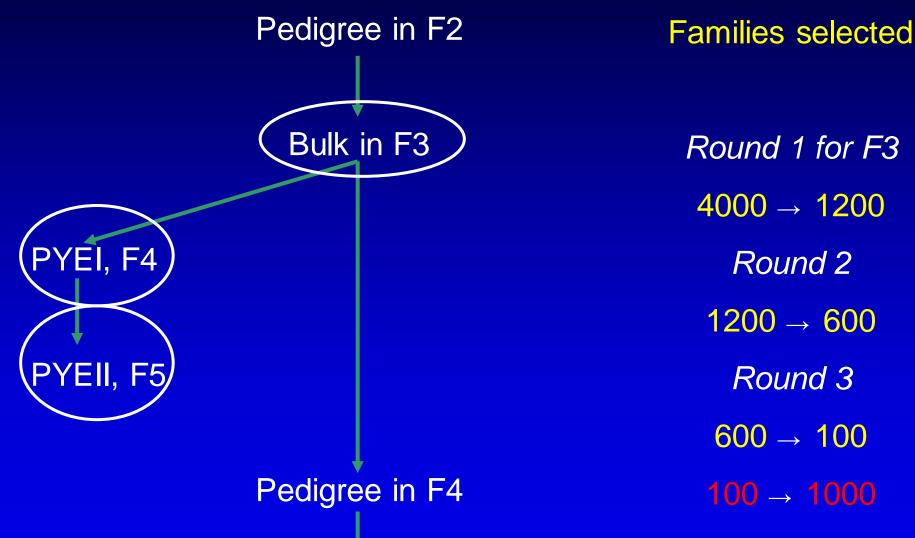
Number of selection rounds in the generation: any integer more than 1

- Seed source indicator
 - 0: Seed for selection round i(i > 1) come from round 1
 - 1: Seed for selection round i (i > 1) come from round i-1

Definition of each selection round



An example (LRC, Toowoomba, Australia) for seed source indicator 1



- Title for the generation
- Seed propagation type (within a family)
 - clone, asexual reproduction
 - DH, doubled haploid
 - self, self-pollination
 - backcross, backcrossed to one parent
 - topcross, crossed with a third parent (three-way cross)
 - doublecross, crossed with another F1
 - random, random mating
 - noself, random mating but self-pollination is eliminated

Generation advance method (or harvest method): management of the selected individual plants in a family

- pedigree: the selected plants in each family are harvested individually, resulting a few families in the next generation
- bulk: the selected plants in each family are harvested in bulk, resulting one family in the next generation

Field experiment design

- Number of replications for each family
- Number of plants in each replication
- Number of test locations
- Environment type for each test location
 - defined in the GE system
 - if 0, randomly determined based on environment frequency

>Among family and within family selection

- Number of traits used for selection
- Definition of each trait

Definition of each trait used in selection

- Trait number, for the trait in selection (0 when marker score is used in selection)
- Selection mode
 - T for top, e.g. yield, tillering, grains per spike and 1000-kernel weight
 - B for bottom, e.g. lodging and rusts
 - M for middle, e.g. height and heading
 - R for random, for some special studies
 - TV for top value
 - BV for bottom value
 - TN for a number of individuals/families with top phenotypic values
 - BN for a number of individuals/families with bottom phenotypic values
 - RN for a number of individuals/families to be selected randomly

Selected proportion or value: the proportion or value of individual plants in a family (for within family selection) or of families (for among family selection) to be selected **Proportion selection**

Threshold selection

Number selection

An example of generation definition

Rounds of selection	Seed source indicator	Generation title	Seed propagation type	Generation advance method	Replicat ions	Plot size	Test locations	Environment type	
1	0	F6	self	pedigree	1	750	1	2, Toluca	
4	0	F7	self	bulk	1	70	1	1, Obregon	
		F8(T)	self	bulk	1	70	1	2, Toluca	
		F8(B)	self	bulk	1	70	1	3, El Batan	
		F8(YT)	self	bulk	1	100	1	1, Obregon	
1	0	F8(SP)	self	bulk	1	30	1	1, Obregon	

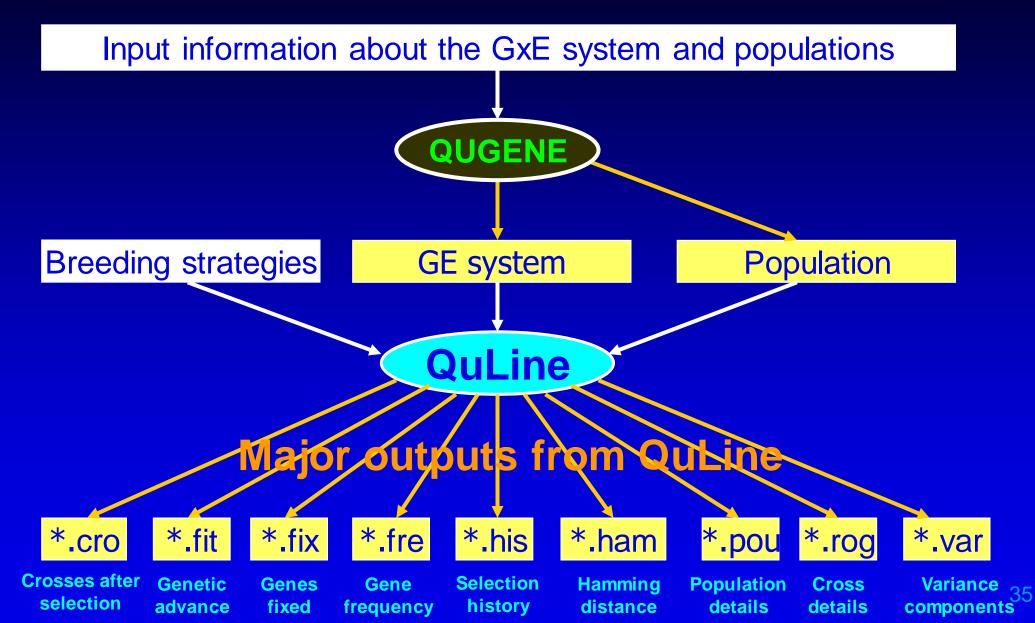
Traits, their selection modes and selected proportions

Trait	Yield	Lodg- ing	Stem rust	Leaf rust	Stripe rust	Height	Tillering	Heading	Grains per spike	1000 kernel weight	Total
Selection mode	т	В	В	В	В	Μ	т	М	т	т	
F6, among		0.99			0.96		0.95				0.90
F6, within		0.90			0.70	0.90	0.95	0.98	0.10		0.05
F7, among		0.85		0.70		0.98	0.85	0.96	0.70	0.75	0.25
F8(T), among		0.55			0.70	0.99	0.98	0.99	0.90		0.55
F8(B), among				0.90							0.90
F8(YT), among	0.40										0.40
F8(SP), among											1.00

In QuLine, a breeding program looks like ...

! NR	ss	GT	PT	GA	RP PS NL ET Row 1
!				AT	(ID SP SM) Row 2
!				WT	(ID SP SM) Row 3
1	0	CB	self	bulk	1 10 1 2
				0	
				0	
1	0	F1	singlecross		1 20 1 1
				7	2 в 0.98 3 в 0.99 4 в 0.85 6 м 0.99 7 т 0.90 8 в 0.98 9 т 0.97
				0	
1	0	F2	self	pedig	ree 1 1000 1 2
				7	
					2 B 0.95 4 B 0.99 5 B 0.40 6 M 0.85 7 T 0.60 8 B 0.90 9 T 0.50 10 T 0.60
1	0	F3	self	bulk	
				7	2 в 0.90 3 в 0.99 4 в 0.70 6 м 0.97 7 т 0.75 8 в 0.95 9 т 0.80
					4 B 0.90 6 M 0.95 8 B 0.95 9 T 0.30 10 T 0.60
1	0	F4	self	bulk	
					2 B 0.90 5 B 0.65 6 M 0.97 7 T 0.85 8 B 0.97 9 T 0.85
	_	_			5 B 0.90 6 M 0.95 8 B 0.95 9 T 0.30 10 T 0.60
1	0	F5	self	bulk	
					2 B 0.90 4 B 0.75 6 M 0.97 7 T 0.85 8 B 0.95 9 T 0.85
				5	4 B 0.90 6 M 0.95 8 B 0.95 9 T 0.30 10 T 0.60
1	0	F6	self		pree 1 140 1 2
				6	
	•		1.0	5	5 B 0.90 6 B 0.90 7 T 0.95 8 B 0.95 9 T 0.10
4	0	F7	self	bulk	
				7	2 в 0.90 4 в 0.75 6 м 0.97 7 т 0.90 8 в 0.95 9 т 0.85 10 т 0.75
		AT (m)	16	0	1 70 1 0
		AL(T)	self	bulk 6	1 70 1 2 2 в 0.95 5 в 0.90 6 м 0.99 7 т 0.98 8 в 0.99 9 т 0.85
				0	2 B 0.95 5 B 0.90 6 M 0.99 7 T 0.98 8 B 0.99 9 T 0.85
		AL(B)	self	bulk	1 70 1 3
		АП (Р)	Sell		4 B 0.90
				0	
		PYT	self	bulk	1 100 1 1
		FII	Serr		1 T 0.40
				0	

Steps to run QuLine

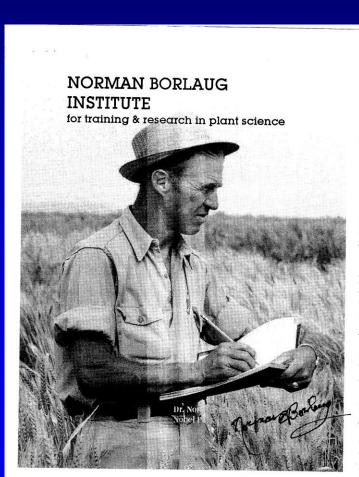


Comparison of two breeding strategies: modified pedigree (MODPED) and selected bulk (SELBLK)

> Crop Science, 2003, 43: 1764-1773 Crop Science, 2004, 44: 2006-2018

Dr. Borlaug and the Green Revolution

- Mexican-Rockefeller Foundation Agricultural Program, 1944
- Mexico became self-sufficient in most food commodities by the late 1950s
- Young scientists were trained in Mexico
- Semidwarf wheats (*Rht1* gene and Norin 10)
- 1965-1975, wheat and rice production had increased by 50%
- > Awarded Nobel Peace Prize in 1970



CIMMYT's Shuttle Breeding

November to May

Obregon, 27° N, 39 masl. 8-11 t/ha under

May to November El Batan Toluca, 19º N, 2640 masl. High rainfall (800-900 mm)

Breeding methods with self-pollinated crops

Allard, R.W. 1960. Principles of plant breeding. John Wiley & Sons, Inc.
Stoskopf, N.C. 1993. Plant Breeding — Theory and

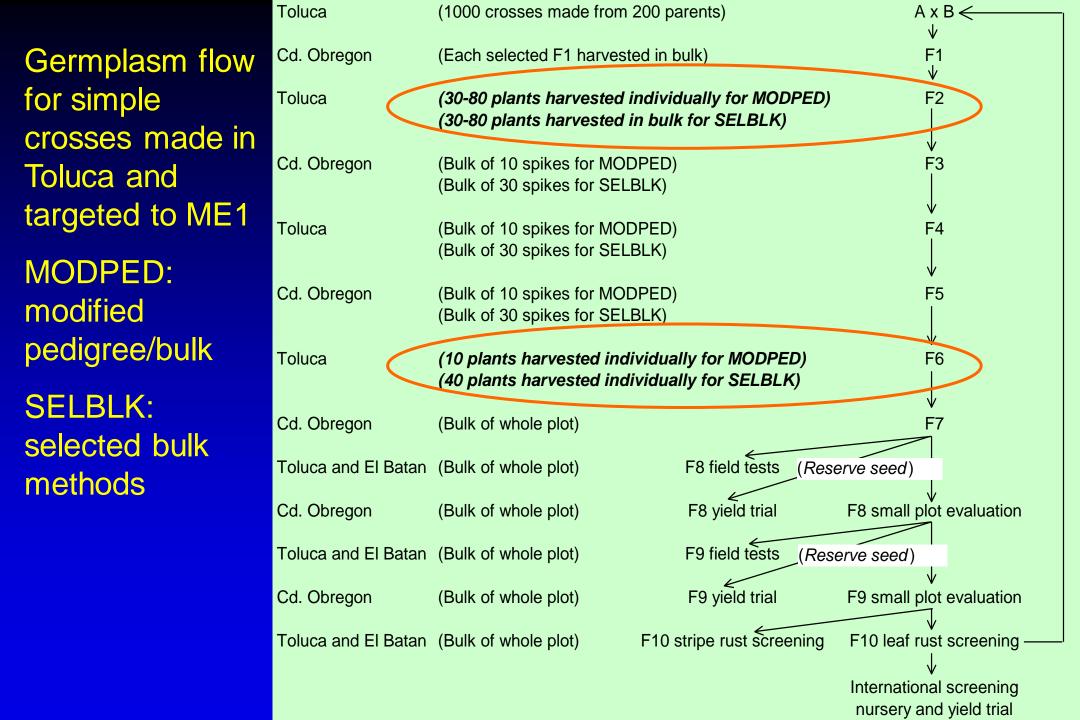
Practice. Westview Press.

- Mass and pure-line selection
- The pedigree system
- The bulk population method
- The backcross breeding method
- Single seed descent (SSD), a special case of pedigree system
- Recurrent selection breeding method
- Mutation breeding
- Haploid breeding system (doubled haploid)

Breeding methods in CIMMYT's wheat breeding program

Pedigree system: before 1984.

- "Pedigree selection" is used from F2 to F6.
- Modified pedigree/bulk (MODPED): in 1985-1989/94.
 - "Pedigree selection" is used in F2 and F6, and "bulk selection" is used in other generations.
- Selected bulk (SELBLK): after 1995.
 - "Pedigree selection" is used only in F6, and "bulk selection" is used in other generations.



Trait, segregating gene number, gene effects and trait heritability

Trait	Genes	Gene effect type	AA	Aa	aa	Trait range	h _b ² (Indiv. plant)
Yield	20, 40	E0, E1, E2	Random v	value from l	UD (0, 1)		0.05
Lodging	3	additive	0	5	10	0-30	0.10
Stem rust	5	additive	0	0.5	1	0-5	0.30
Leaf rust	5	additive	0	5	10	0-50	0.30
Yellow rust	5	additive	0	5	10	0-50	0.30
Height	3	additive	40	30	20	120-60	0.45
Tillers/plant	3	additive	5	3	1	15-3	0.35
Heading	5	additive	20	16	12	100-60	0.30
Grains/spike	5	additive	14	10	6	70-30	0.35
Seed weight	5	additive	12	8.5	5	60-25	0.35

Trait correlation and pleiotropy

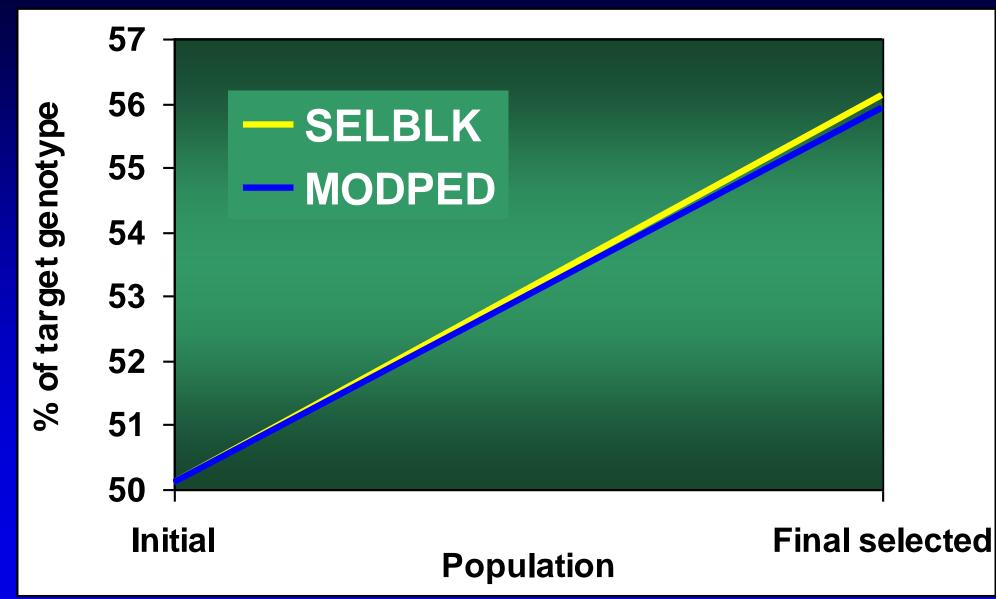
Trait	Yield	Lodging	Stem rust	Leaf rust	Yellow rust	Height	Tillers/ plant	Heading	Grains/ spike	Seed weight
Yield	******	-0.50	-0.20	-0.10	-0.10	-0.50	0.40	0.30	0.50	0.40
Lodging	-0.56	and the second second								
Stem rust	-0.25		******			Es		ed by		
Leaf rust	-0.05			***********		CIMN	1YT k	oreede	rs	
Yellow rust	-0.09				*******	******				
Height	-0.62						****			
Tillers/plant	-0.08	Estil	mate	ed fr	om ti	he		****	-0.20	-0.40
Heading	0.60	define	ed ge	enet	ic mo	odel				
Grains/spike	0.09						-0.17		********	-0.30
Seed weight	-0.07						-0.30		-0.07	********

Experiment design

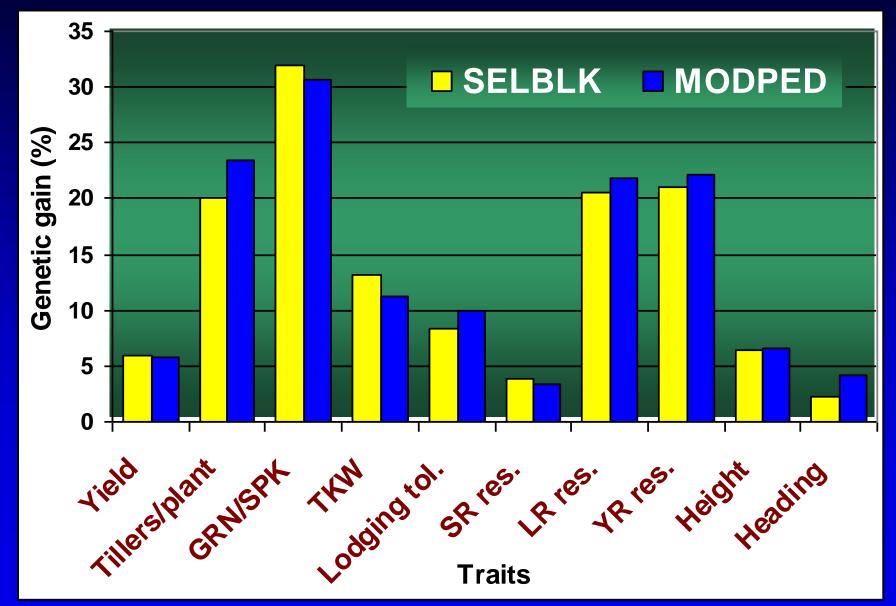
> 12 Genotype and environment (GE) systems

- Two yield gene numbers: 20 and 40, two alleles for each gene
- Pleiotropy (same gene effects various traits): absent and present
- Epistasis (multiple gene interaction): no epistasis, digenicepistasis, and tri-genic epistasis
- Linkage: no linkage (independent gene segregation)
- Initial population
 - 200 parents, gene (allele) frequencies of 0.5 for all genes
- > 1000 crosses were made
- >258 lines were selected after 10 generations of selection

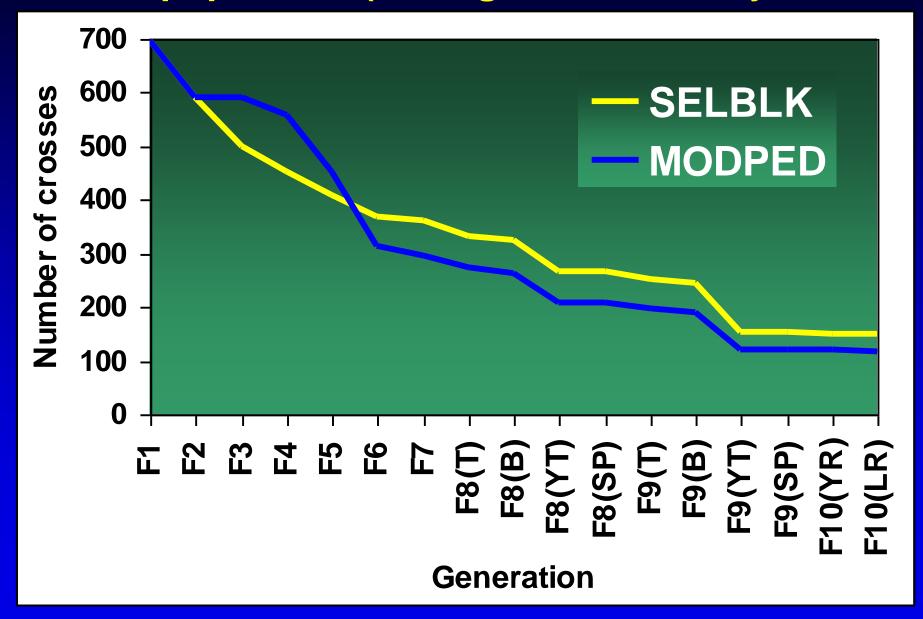
Result 1: Genetic gain in yield from SELBLK is 3.3% higher than MODPED. SELBLK is slightly more efficient.



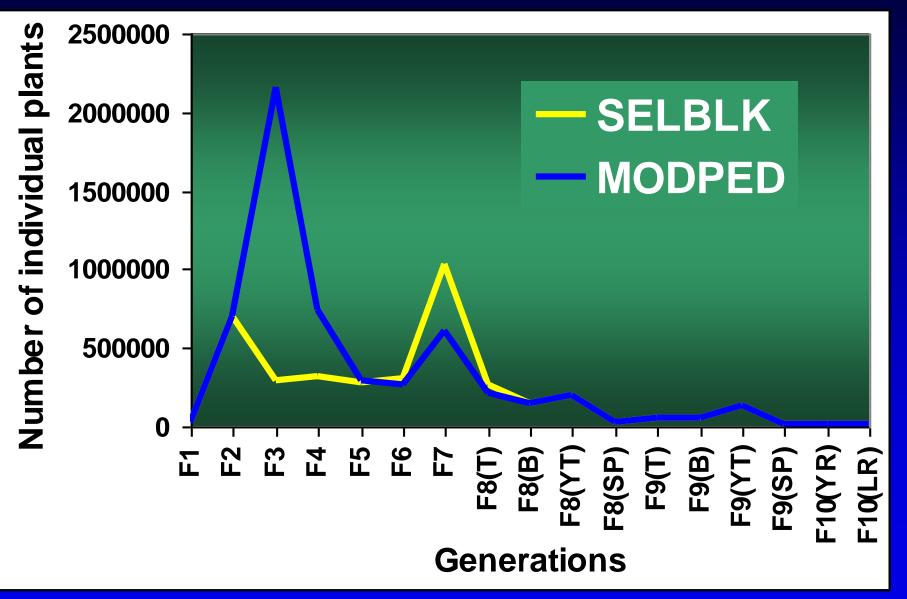
For gains per spike and 1000-kernel weight, SELBLK has a faster genetic gain. For tillers/plant, MODPED has a faster genetic gain.



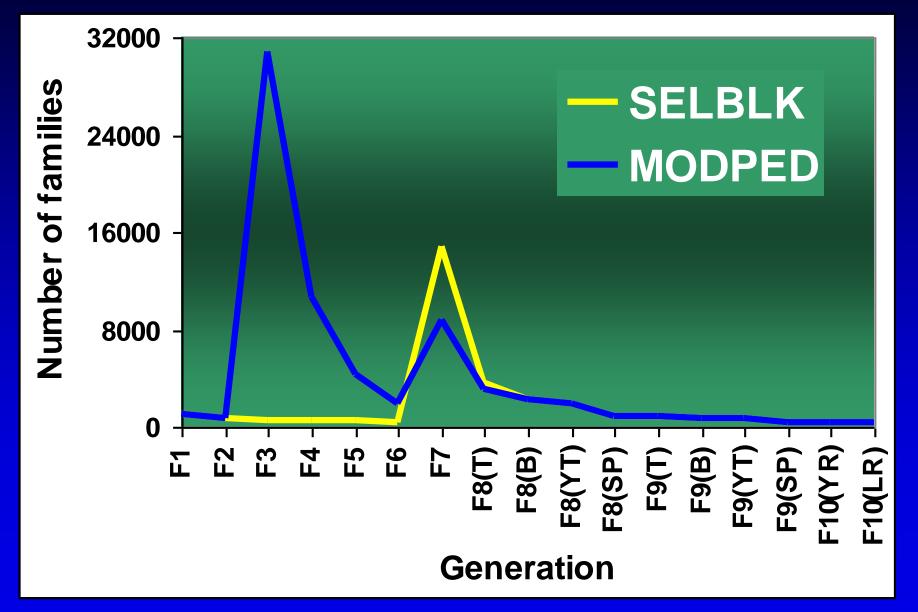
Result 2: SELBLK retained 25% more crosses in the final selected population (more genetic diversity retained)



Result 3: SELBLK required 1/3 less land from F1 to F8 than MODPED. SELBLK is more cost-effective.



Result 4: SELBLK produced 40% less families (plots) to be planted from F1 to F8 (less labor required)



Modeling of the Single Backcrossing Breeding Strategy (SBBS)

Theor. Appl. Genet., 2009, 118: 683-694

Ravi Singh, wheat breeder and pathologist in CIMMYT



Adapted wheats X durable disease resistance donors 1-2 times of backcrossing with the adapted Large BCF2 population **Select** adapted lines combined with durable resistances

Estimated percentages of favourable alleles or gene combinations in different parental lines in wheat breeding at CIMMYT

Category	favorable genes (%)	Example	% total lines
Elite adapted lines (EAL)	80-85	Major released cultivars in targeted mega- environments (MEs) either developed by CIMMYT or by partners	10
Adapted lines (AL)	75-80	Elite advanced lines from CIMMYT's International Nursery and Yield Trials	60
Intermediate adapted lines (IAL)	65-75	Advanced lines from CIMMYT's Yield Trials in Ciudad Obregón and Toluca, Mexico	10
Un-adapted (or non- adapted) lines (UAL)	20-40	Land races	2
Second generation of re- synthesized wheat (SYNII)	40-60	Derived lines between the first generation of re-synthesized wheat derivatives and adapted lines	10
First generation of re- synthesized wheat (SYNI)	20-40	Derived lines between primary re-synthesized wheat and adapted lines	5
Primary re-synthesized wheat (SYN0)	0-30	Inter-specific crosses between <i>Triticum durum</i> and <i>Aegilops tauschii</i>	3

Two traits defined in QU-GENE

Adaptation

- 200 genes on the 21 wheat chromosomes
- Lowest adaptation with no favorable alleles: 0
- Highest adaptation with all favorable alleles: 100Heritability: 0.5
- Donor traits (DT) to be transferred
 - 10 genes governing the donor traits
 - Lowest DT with no favorable alleles: 0
 - Highest DT with all favorable alleles: 10
 - Heritability: 0.5

Adapted parental groups in simulation

Gene frequency of favorable adaptation alleles fixed at 0.8

A0: the frequency of favorable DT alleles is 0
A2: the frequency of favorable DT alleles is 0.2
A4: the frequency of favorable DT alleles is 0.4
A6: the frequency of favorable DT alleles is 0.6
A8: the frequency of favorable DT alleles is 0.8

Donor parental groups in simulation

Gene frequency of favorable DT alleles fixed at 1.0

 \succ D0: the frequency of favorable adaptation alleles is 0 \geq D1: the frequency of favorable adaptation alleles is 0.1 \succ D2: the frequency of favorable adaptation alleles is 0.2 > D3: the frequency of favorable adaptation alleles is 0.3 > D4: the frequency of favorable adaptation alleles is 0.4 \geq D5: the frequency of favorable adaptation alleles is 0.5 \geq D6: the frequency of favorable adaptation alleles is 0.6 > D7: the frequency of favorable adaptation alleles is 0.7

Crosses made between different parental groups for wheat breeding at CIMMYT

Category	Percentage of total crosses	Similarity to defined parental groups
(EAL+AL) $ imes$ (EAL+AL)	65	(A0+A2+A4+A6+A8) ×D7
(EAL+AL) $ imes$ IAL	10	(A0+A2+A4+A6+A8) ×D5+D6)
(EAL+AL) $ imes$ UAL	5	(A0+A2+A4+A6+A8) ×(D2+D3+D4)
(EAL+AL) $ imes$ SYNII	10	(A0+A2+A4+A6+A8) ×(D6+D7)
(EAL+AL) $ imes$ SYNI	7	(A0+A2+A4+A6+A8) ×(D4+D5)
(EAL+AL) $ imes$ SYN0	3	(A0+A2+A4+A6+A8) ×(D0+D1+D2)

The single backcrossing breeding strategy (SBBS)

Generation	Seed propagation method	No. crosses or families grown	Individuals per cross or family	No. selected crosses or families	No. selected individuals in each cross or family
F ₁	Hand pollination between adapted and donor lines	100	20	100	20
B_1F_1	Backcrossing to the adapted parents	100	400	100	50
B_1F_2	Selfing	100	1200	100	30
B_1F_3	Selfing	100	400	100	10
B_1F_4	Selfing	100	400	100	10
B_1F_5	Selfing	100	400	100	10
B_1F_6	Selfing	1000	200	30	200
Final selecte	ed advanced lined	10			

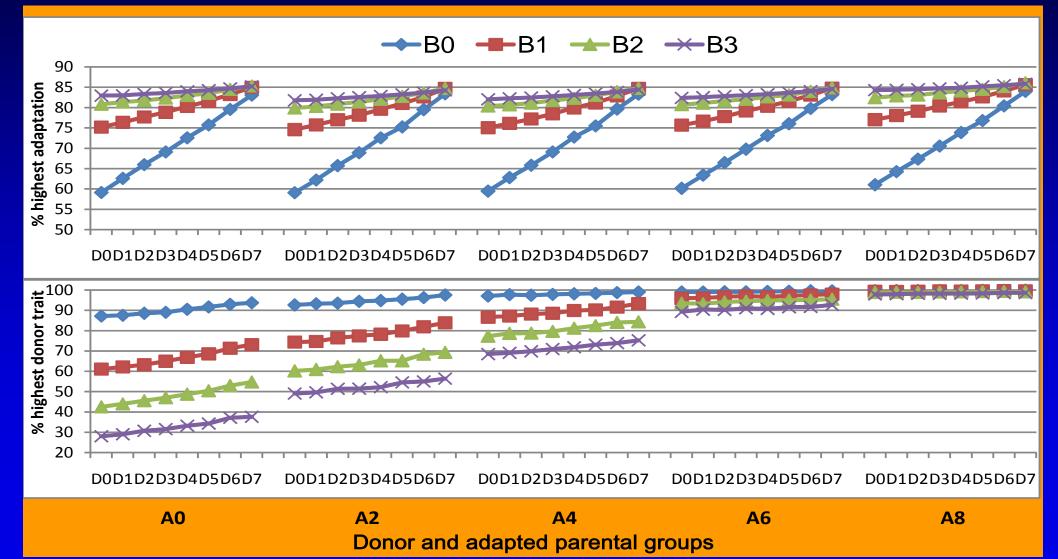
Four crossing strategies defined in QuLine

No backcross	One time of backcross	Two times of backcross	Three times of backcross	Generation advance method
B0	B1	B2	B3	
F ₁	F ₁	F ₁	F ₁	Bulk
F_2	BC_1F_1	BC_1F_1	BC_1F_1	Bulk
F_3	BC_1F_2	BC_2F_1	BC_2F_1	Bulk
F_4	BC_1F_3	BC_2F_2	BC_3F_1	Bulk
F_5	BC_1F_4	BC_2F_3	BC_3F_2	Bulk
F_6	BC_1F_5	BC_2F_4	BC_3F_3	Pedigree
F ₇	BC_1F_6	BC_2F_5	BC_3F_4	Bulk

Six selection schemes

- AD: Adaptation is selected first, followed by the selection for DT
- DA: DT is selected first, followed by the selection for Adaptation
- ADA: Adaptation is selected first, followed by the selection for DT, and adaptation is selected again
- DAD: DT is selected first, followed by the selection for Adaptation, and DT is selected again
- ADAD: Adaptation and DT are selected two times in each generation, and adaptation is selected first
- DADA: Adaptation and DT are selected two times in each generation, and DT is selected first

Genetic advance of selection scheme AD



Conclusions

- We recommend the use of SBBS based on three assumptions:
 - multiple genes governing the phenotypic traits to be transferred from donor parents to adapted parents
 - donor parents still have some favorable genes that may contribute to the improvement of adaptation in the recipient parents even under low adaptation
 - the conventional phenotypic selection is applied or the individual genotypes cannot be precisely indentified

Breeding with known gene information

Aust. J. Agric. Res., 2005, 56: 465-473

Glutenin genes and wheat quality

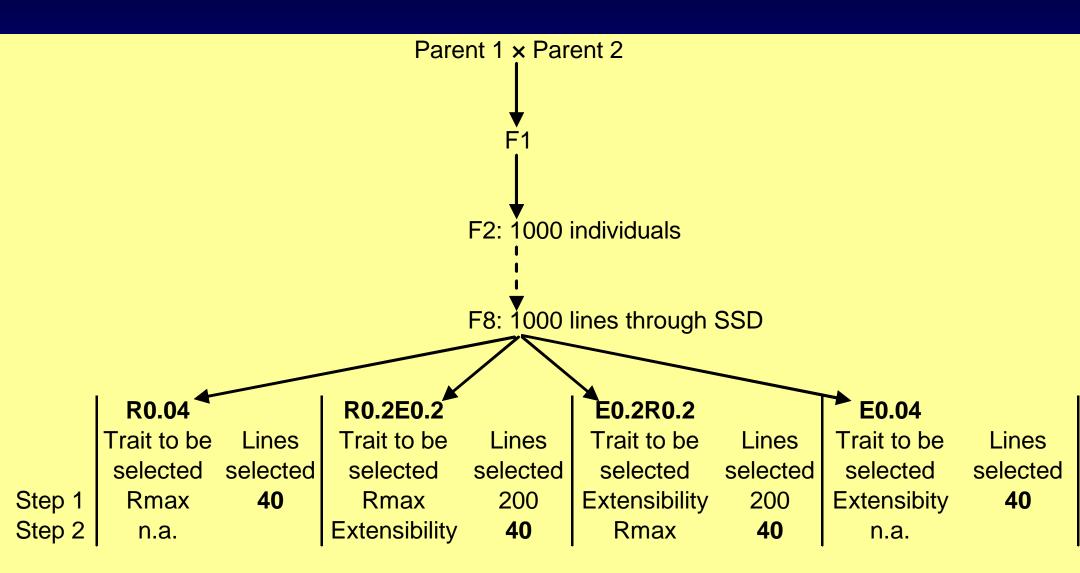
Six glutenin genes

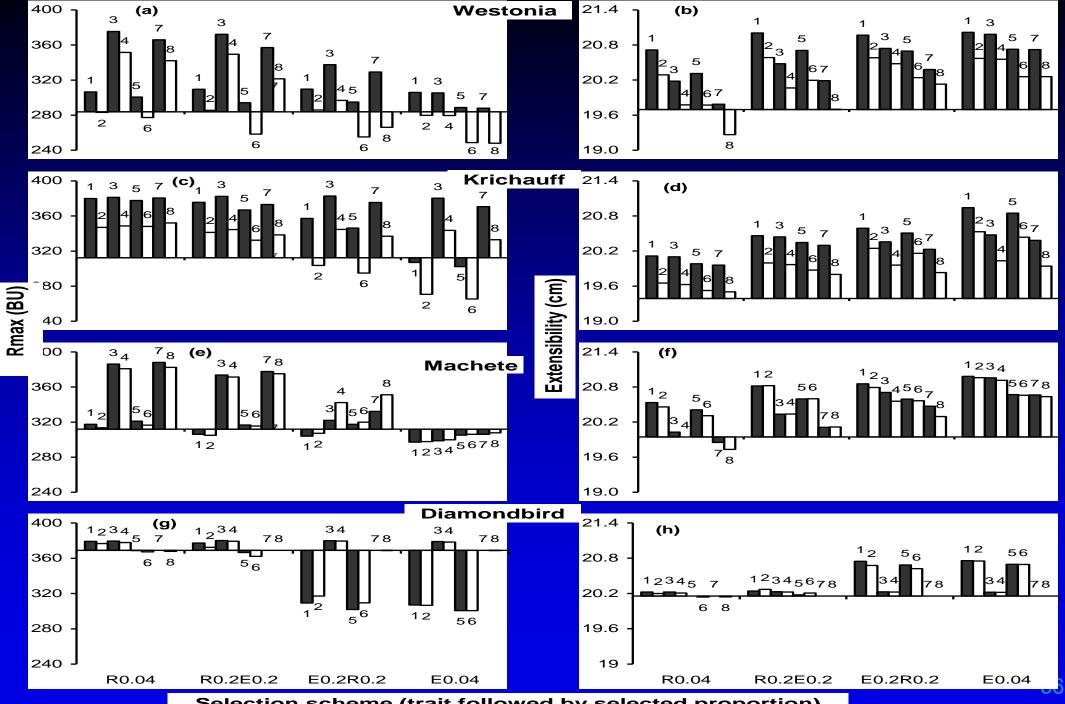
- *Glu-A1* (1AL), *Glu-B1* (1BL), *Glu-D1* (1DL) for HMW
- Glu-A3 (1AS), Glu-B3 (1BS), Glu-D3 (1DS) for LMW
- Two end-use quality traits commonly used by wheat breeders
 - Rmax (BU), for maximum dough resistance
 - Extensibility (cm), for dough extensibility
- Multiple alleles on each gene locus
 - Glu-A1: 1, 2*, and Null
 - Glu-B1: 7, 7+8, 7+9, 6+8, 20, 13+16, 14+15, 17+18, and 23+24
 - *Glu-D1*: 2+12, 4+12, 5+10, and 2+T2

Selected parents

Parent	Rmax	Extensibility
Silverstar 1	309.80	20.78
Silverstar 2	270.20	20.31
Silverstar 7	368.90	20.15
Silverstar 8	329.30	19.68
Westonia	283.70	19.70
Krichauff	312.26	19.39
Machete	312.03	19.95
Diamondbird	368.88	20.16

Four selection schemes





Selection scheme (trait followed by selected proportion)

The best sister lines under each breeding objective and selection scheme

Parent	Objective	R0.04	R0.2E0.2	E0.2R0.2	E0.04
Westonia	High Rmax	3, 7	3, 7	3, 7	1, 3
	High Ext.	1	1, 5	1, 3, 5	1,3,5,7
Krichauff	High Rmax	1,3,5,7	1,3,5,7	3, 7	3, 7
	High Ext.	1,3,5,7	1,3,5,7	1, 5	1, 5
Machete	High Rmax	3,4,7,8	3,4,7,8	4, 8	None
	High Ext.	1,2,5,6	1,2,5,6	1, 2, 3	1,2,3,4
Diamondbird	High Rmax	1,2,3,4	1, 3, 4	3, 4	3, 4
	High Ext.	None	None	1,2,5,6	1,2,5,6

Efficient selection of multiple genes via marker-assisted selection, an example in wheat

Wang, J.,* S.C. Chapman, D.B. Bonnett, G.J. Rebetzke, and J. Crouch. 2007. Application of population genetic theory and simulation models to efficiently pyramid multiple genes via marker-assisted selection. Crop Science 47: 580-588.

Nine major genes to be pyramided in wheat

Gene	Rht-B1	Rht-D1	Rht8	Sr2	Cre1	VPM	Glu-B1	Glu-A3	tin
Chr.	4BS	4DS	2DL	3BS	2BL	7DL	1BL	1AS	1AS
Marker	Codom	Codom	Codom	Codom	Dom	Dom	Codom	Codom	Codom
MK-gene distance	0	0	0.6	1.1	0	0	0	0	0.8
HM14BS	Rht-B1a	Rht-D1a	Rht8	sr2	cre1	vpm	Glu-B1a	Glu-A3e	Tin
Sunstate	Rht-B1a	Rht-D1b	rht8	Sr2	cre1	VPM	Glu-B1i	Glu-A3b	Tin
Silverstar+ tin	Rht-B1b	Rht-D1a	rht8	sr2	Cre1	vpm	Glu-B1i	Glu-A3c	tin
Target	Rht-B1a	Rht-D1a	Rht8	Sr2	Cre1	VPM	Glu-B1i	Glu-A3b	tin

One strategy identified by QuLine to combine the nine genes from topcross

- Selection of Sunstate as the final parent (having largest number of favorable alleles) in the topcross
- Stage I: Selection for *Rht-B1a* and *Glu-B1i* homozygotes, and enrichment of *rht8*, *Cre1*, and *tin* in TCF1
- Stage II: Selection of homozygotes for one target allele, e.g. *Rht8*, and enrich remaining target alleles in TCF2
 Stage III: Selection of the target genotype in DHs/RILs

Comparison with other strategies

For this strategy, one target genotype can be selected by screening < 600 individuals/lines</p>

For one-stage selection in advanced generations, one target genotype can be selected be screening > 3500 lines

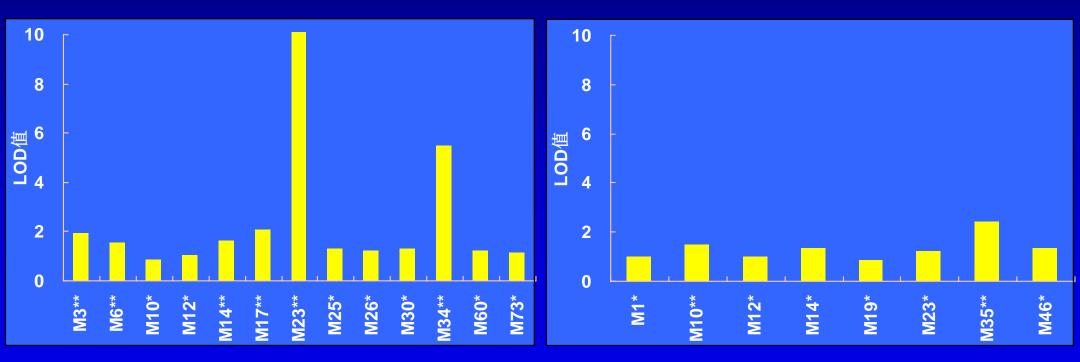
For one-stage selection in early generations, say TCF2, one target genotype can be selected be screening millions of individuals Using QTL mapping results to design the breeding program

Genetical Research, 2006, 88: 93-104 Theor. Appl. Genet., 2007, 115: 87-100

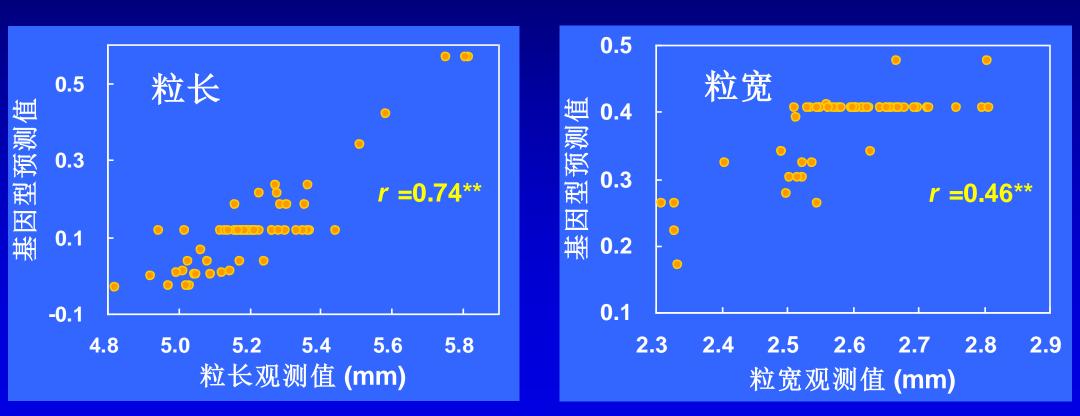
QTL for grain length and grain width in rice using 65 CSS lines

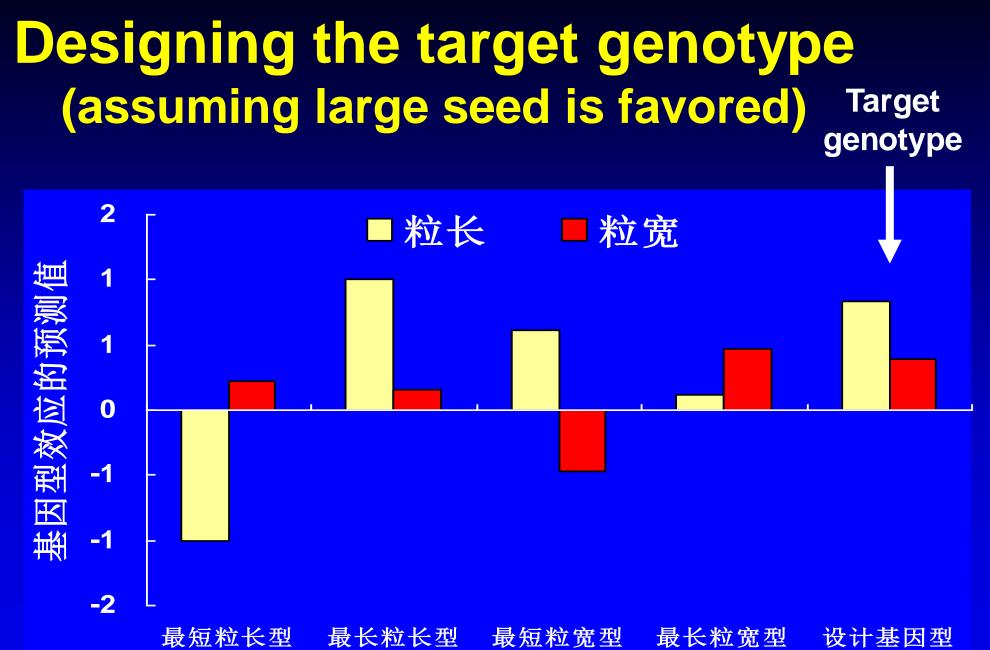
Grain length

Grain width



Prediction using identified QTL





Choosing parental lines

Genotype	M1	M6	M12	M23	M25	Prediction in	
						GL	GW
						(mm)	(mm)
Longest grain	1	2	2	2	2	6.21	2.74
Widest grain	2	1	1	1	1	5.32	3.07
Designed TG	2	2	1	2	2	6.05	2.98
CSSL5	1	2	1	1	1	5.44	3.00
CSSL16	2	1	1	2	1	5.77	2.98
CSSL19	1	1	2	1	2	5.54	2.93

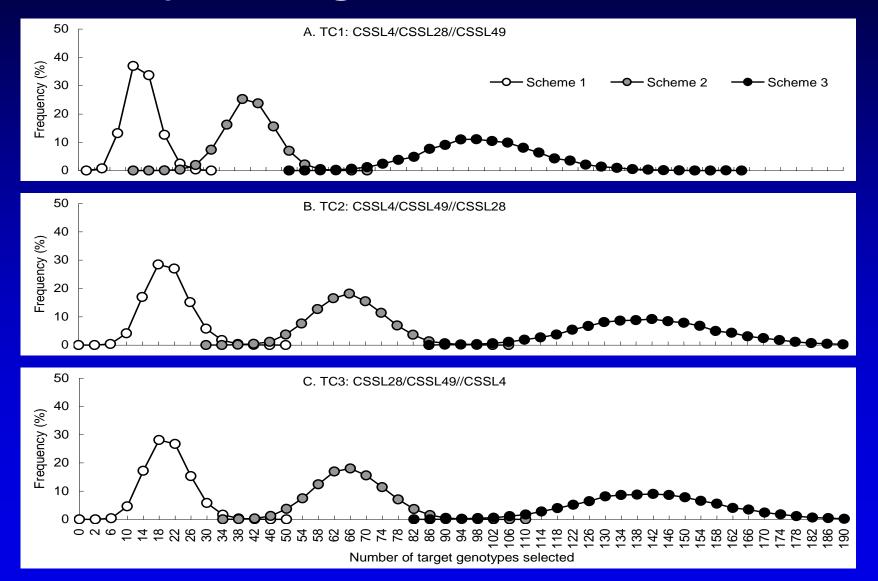
Achieving the designed TG

Three option for crossing TC1: (CSSL5×CSSL16)×CSSL19 TC2: (CSSL5×CSSL19)×CSSL16 TC3: CSSL5×(CSSL16×CSSL19) >Two MAS schemes (just for example) Scheme1: MAS in F8 only Scheme2: MAS in F2 and F8

Choosing the best crossing and selection schemes

TC	MAS	Selected Ind. In F2	F8 families before MAS	F8 families after MAS (S.E.)	DNA samples	DNA samples per TG
TC1	Scheme1	100	3000	7.6 (3.27)	3000	395
	Scheme2	12.0	359	7.6 (3.37)	459	60
TC2	Scheme1	100	3000	24.3 (7.06)	3000	123
	Scheme2	24.8	745	23.3 (7.16)	845	36
TC3	Scheme1	100	3000	11.2 (5.45)	3000	268
	Scheme2	7.5	226	12.3 (5.14)	326	26

Frequency distribution of the number of DG1 from 1000 simulations (Breeding is science, art and chance!)



Breeding is a tedious and long procedure when considering the nature of complex inheritance: linkage, epistasis, pleiotropy, GbyE, etc.

Dr. Borlaug spent more than 20 years from

this tall wheat to this semi-dwarf wheat, which triggered the Green Revolution!





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Demonstration of QuLine