Breeding Strategy and use of HTPG in the CIMMYT global wheat program (GWP)

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Forward Breeding for accelerated crop improvement workshop

August Nov 28-30, 2016

ICRISAT

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Centralized breeding program in Mexico

- ✓ Durum and spring bread wheat program in Mexiko
- ✓ Winter wheat program in Turkey
- ✓ No of crosses
- ✓ Shuttle breeding within Mexico and to Kenya
- ✓ Preliminary yield trails in multiple simulated environments in Mexico

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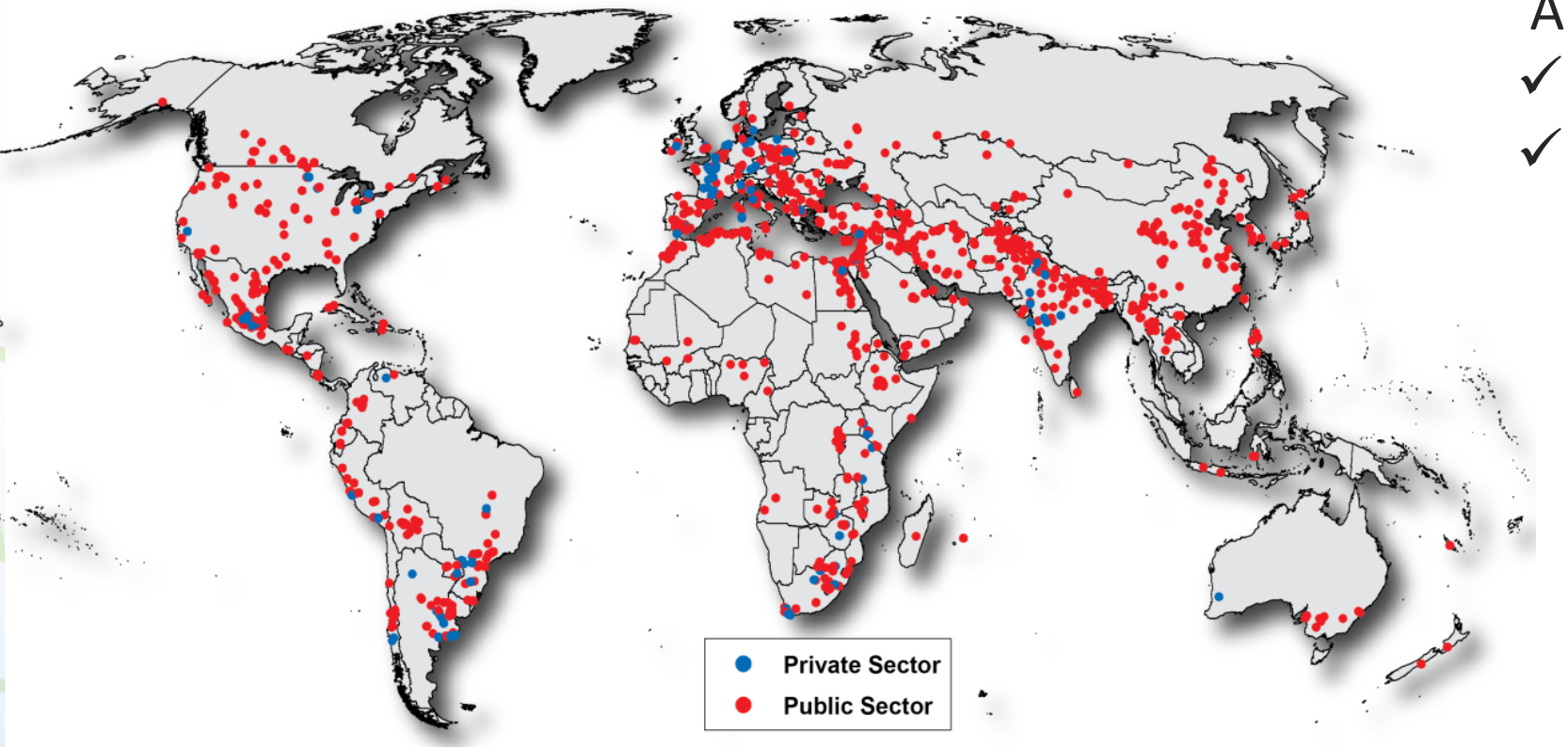
Germplasm distribution via IWIN (International Wheat Improvement Network)



Annual seed shippments:

- ✓ approx. 200 000 wheat lines
- ✓ approx. 450 breeding programs

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Centralized breeding program in Mexico

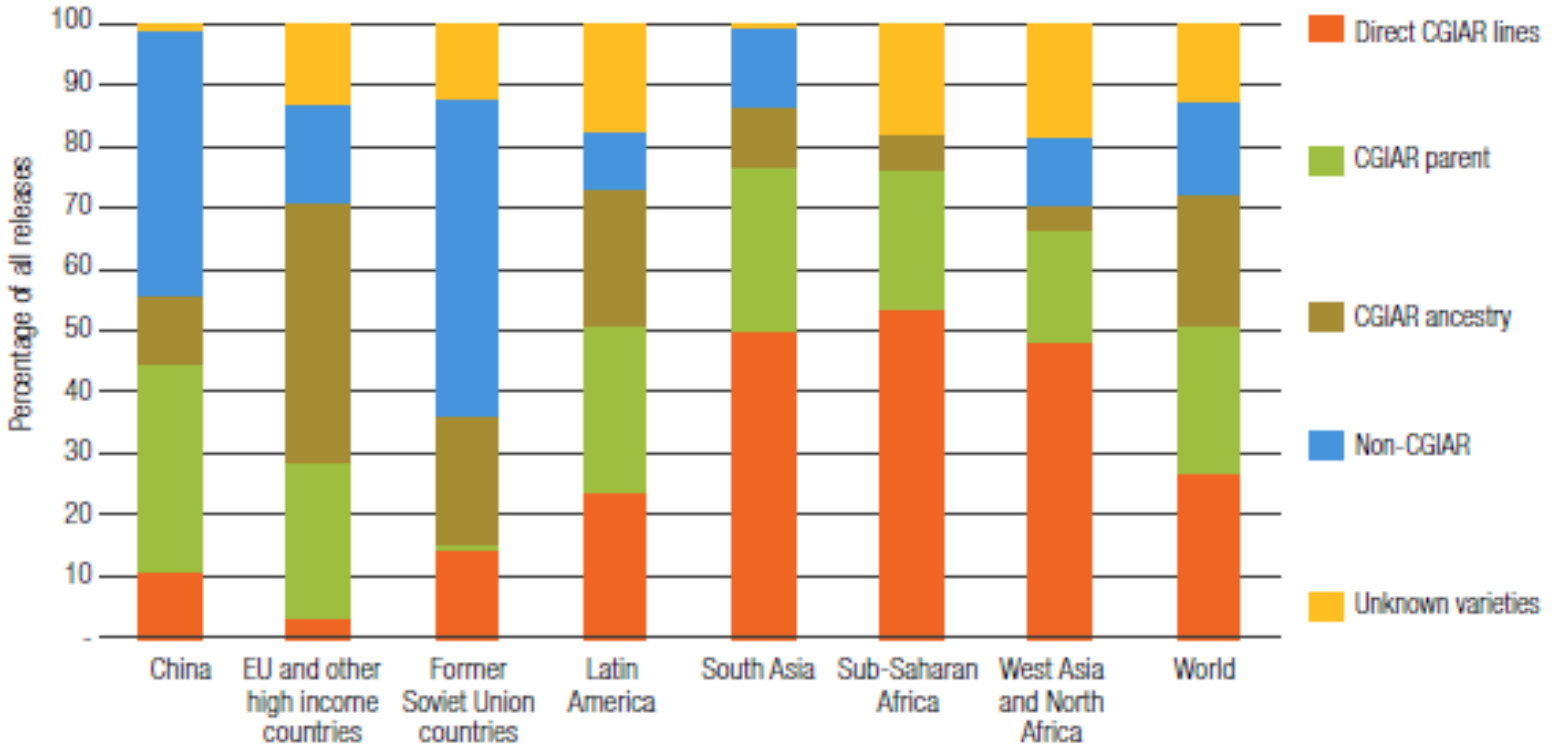

Figure 1. Most spring bread wheat varieties released in developing countries during 1994-2014 are CIMMYT/ICARDA-related; results for spring durum wheat are similarly impressive.

Source: Lantican et al. 2016.

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Germplasm Target traits

Core traits

- ✓ High and stable yield potential
- ✓ Durable resistance to Rusts- Stem (Ug99), Stripe and Leaf
- ✓ Water use efficiency/Drought tolerance
- ✓ Heat tolerance
- ✓ Appropriate end-use quality
- ✓ N-use efficiency

Additional traits for specific mega-environments

- ✓ Durable resistance to diseases and pests
  - Septoria leaf blight
  - Spot Blotch
  - Tan Spot
  - Fusarium head scab and mycotoxins
  - Karnal bunt
  - Aphids
  - Root rots and nematodes
- ✓ Enhanced Zn and Fe concentration

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Marker-based selection strategies

Traits controlled by a few genes with large effects	*Traits controlled by many genes with small effects*
Reductionistic - gene based	Holistic - genome wide
Line evaluation, MABC, MAS	Genomic Prediction
Discover relevant genes	Identify a prediction problem
↓	↓
Identify and validate effects of favorable alleles	Estimate the effects for all genomic regions
↓	↓
Introgression into elite breeding pools	Employ prediction model for a improved or rapid enhancement of breeding values

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Marker-based selection strategies - overview

Gene discovery and marker validation phase

Based on QTL and GWAS studies, public databases and information

Genomic selection

Marker deployment phase

Reductionist approach

- BW – MAS/MABC – rust, insects, nutritional quality
- DW – MAS/MABC – rust, quality
- PT – MAS/MABC – adaptive/agronomic traits
- WC – MAS/MABC – rust, crossability, hybrid necrosis
- PA – Trait-based genotyping
- WW – Trait-based genotyping

Holistic approach

- BW and PT – F5 to F7 prediction

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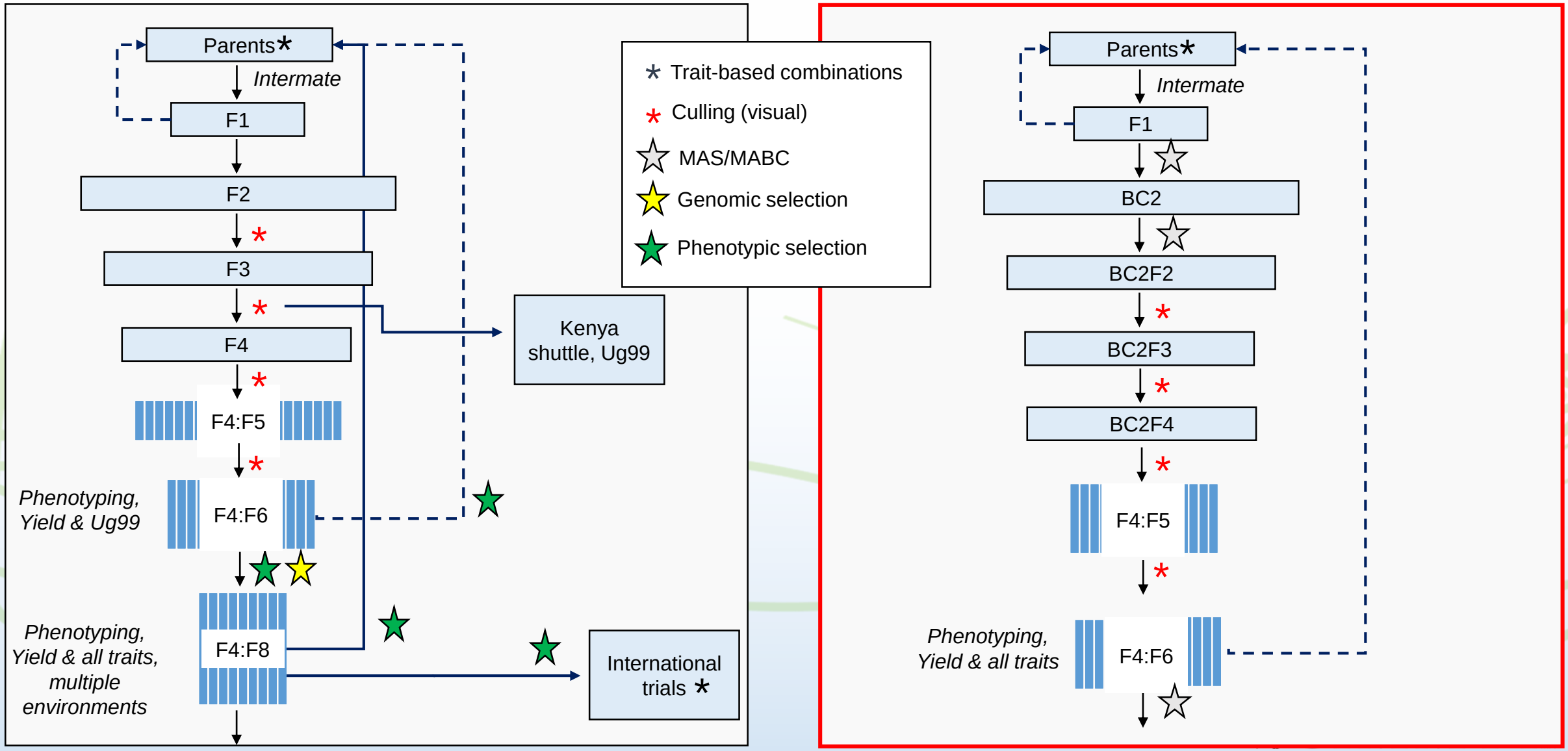
BW: Marker based selection strategy - Current Breeding Cycle



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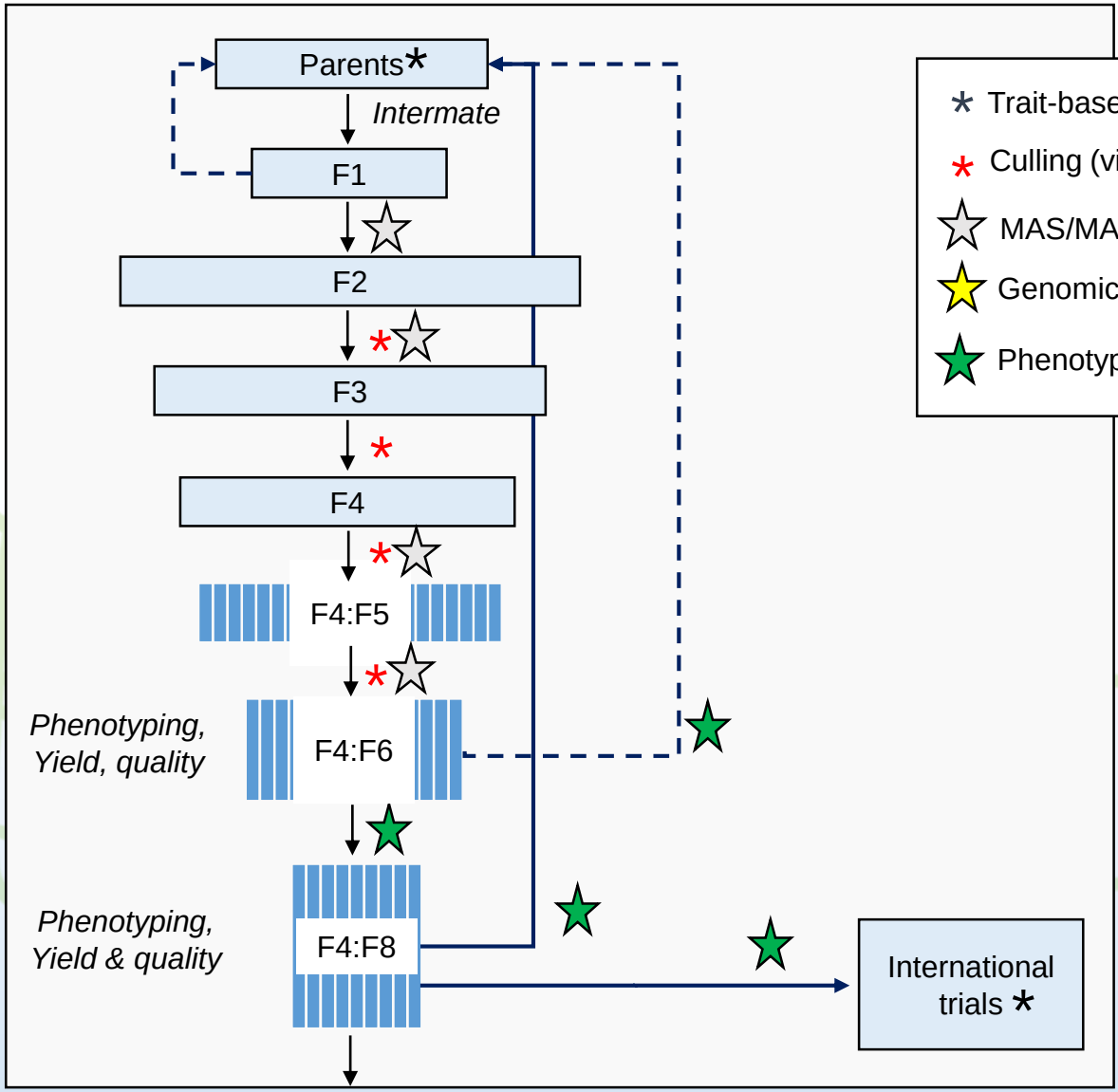
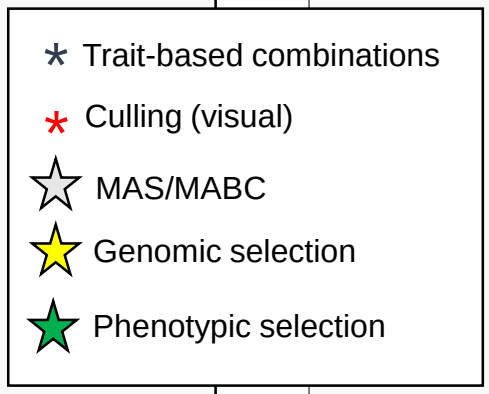
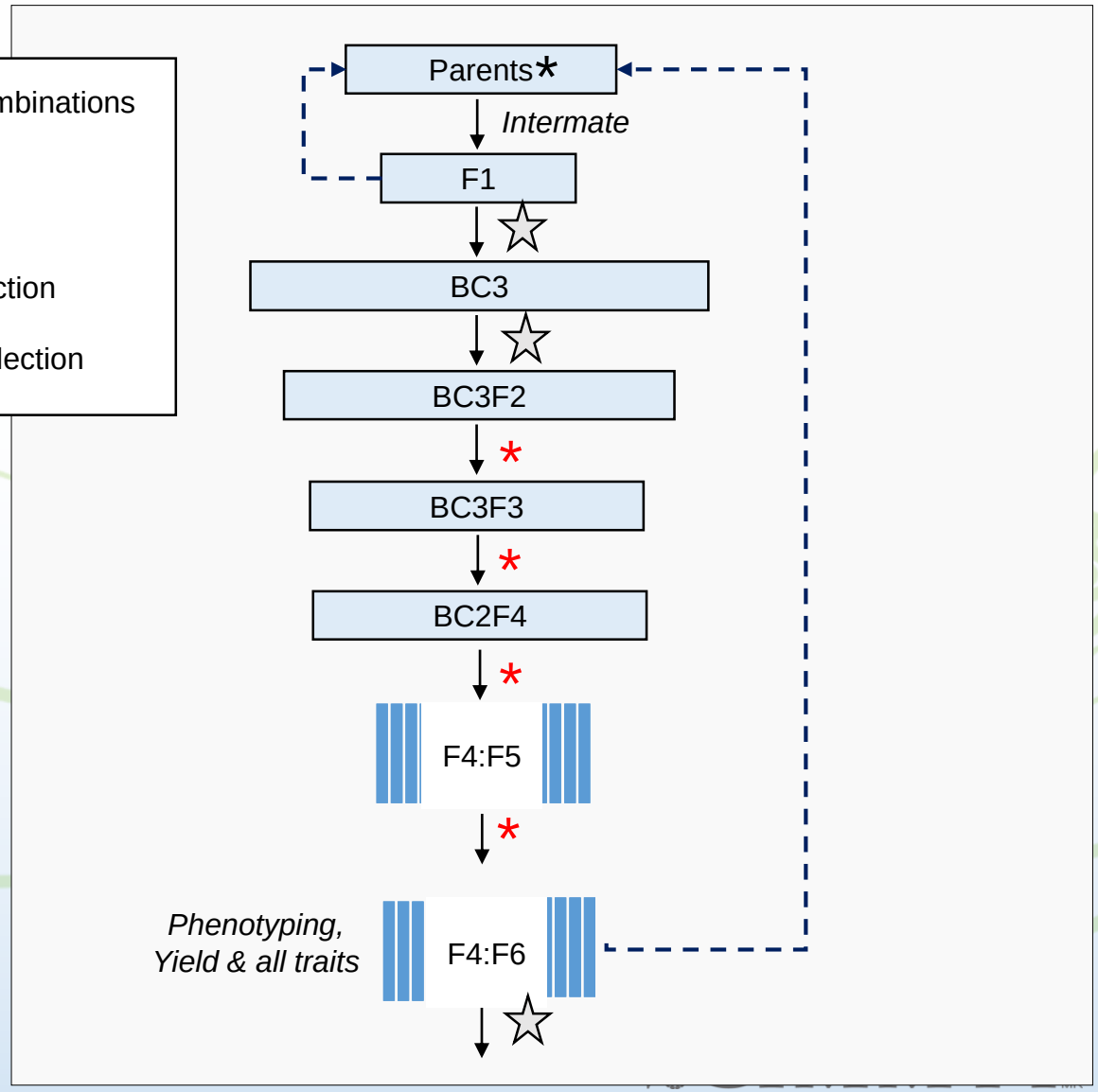
DW: Marker-based selection strategy - Current Breeding Cycle







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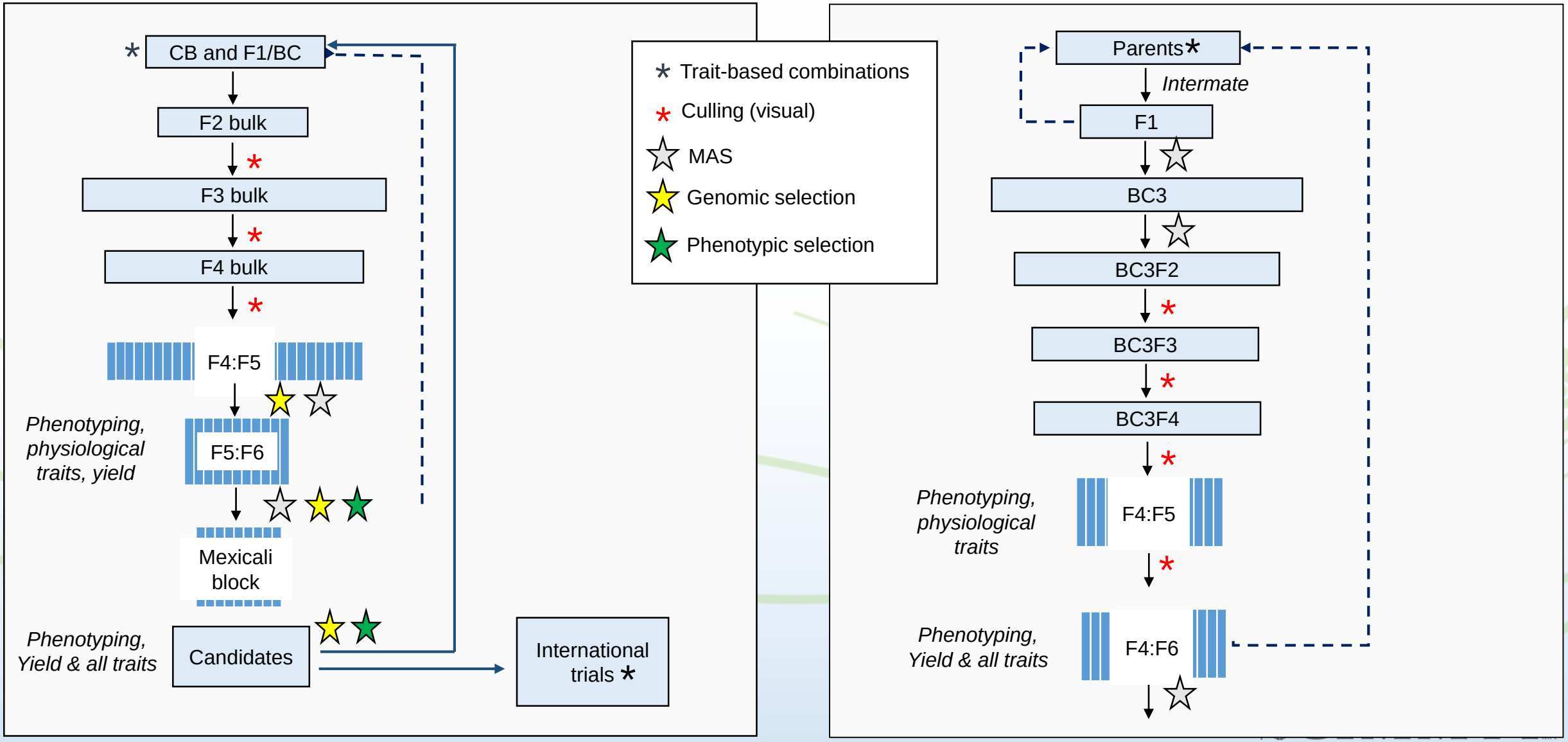
PT: Marker-based selection strategy - Current Pre-breeding Cycle



Case study - CIMMYT GWP

Marker deployment in 2016 (Jan – Sept 2016)

Trait-based marker data points: 160,930

DNA extraction: 18,600 lines

Number of markers/line: 8.6

Percent SNP markers: 71.8

Percent STS/SSR markers: 28.2

Percent SNP markers outsourced: 26.2

SNP array/NGS data points: >750 000

DNA extraction: 9,994 lines

In-house cost:

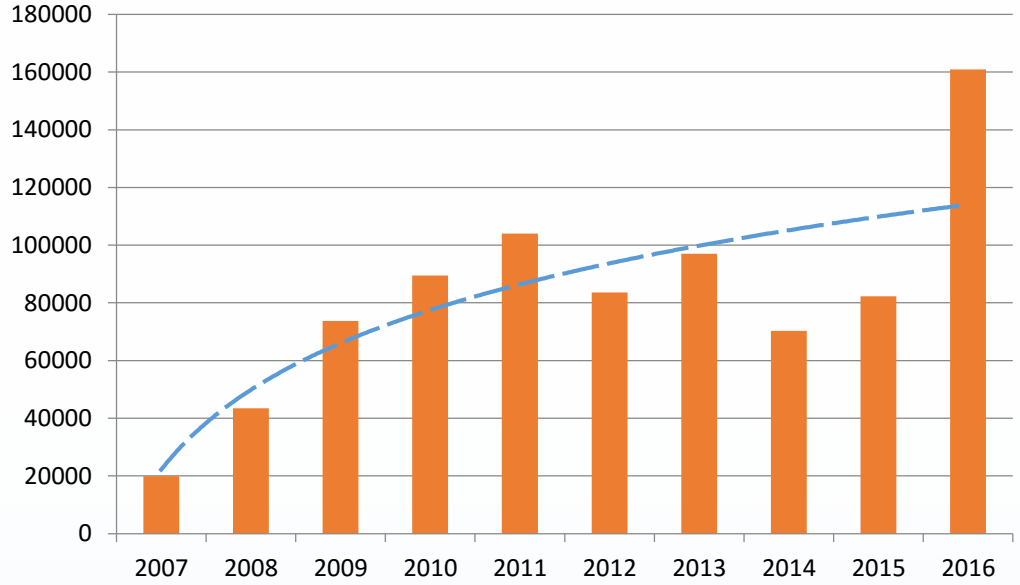
DNA extraction:1.5 USD

STS marker data point 0.8 USD

SNP marker data point 0.3 USD

→ *DNA extraction + 10 SNP: 4.5 USD*

Trait-based marker data points: 2007 - 2016



Gene targets: 2016 -

Program	Trait category	Genes
BW	Rust resistance, Aphid resistance, Zn/Fe concentration, Industrial quality, Nematodes, Aluminium tolerance,	*Lr67, Sr22, Sr26, Sr32, Sr47, Sr50, SrND643, Yr5, Yr15, Yr39, Yr41, Yr51, Yr52, Yr57, Yr59, Yr60, Sr2/Fhb1, Pina-D1a, Alt1, Alt2, wbm, Gba, QTLZn-2B, QTLZn-3A, QTLZn-4B, QTLFhb-2Dc*
DW	Rust resistance, Aphid resistance, Nematodes, Quality	*Lr14a, Lr19/Sr25, Lr37/Yr17/Sr28, Lr47, LrQTL, Sr22, Sr39, H25, Cre1, Fht1/2, Gpc-B1, Glu-1, TaGW2*
PT	Rust resistance, Photoperiod, Vernalization, Earliness *per se*, TGW	*Lr34, Lr46, Lr67, Lr37/Yr17/Sr28, Sr2, Vrn-1, Vrn-3, Ppd-1, Ppd-2, Ppd3, Eps-D1, TaGw2*

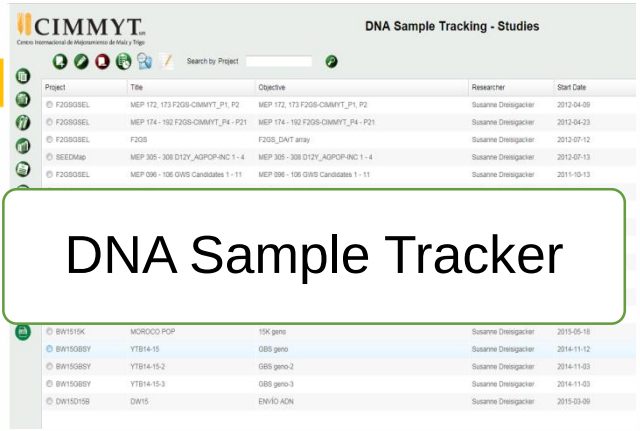
Case study - CIMMYT GWP

Current Logistics

Please help create your project name(s) by selecting from the options below or enter a project name if you already have one from a previous season, previous batch, etc.

	Program	Two-digit year when "project" started	Genotyping purpose	Scientist responsible for project	Batch	Project name	No. of samples	Starting date	Data due by?
Example	*Bread Wheat - BW*		*16Marker-assisted selection -MS*	*Singh, Ravi - BL*	*02*	*BW16MSBL-B02*			
Project 1									
Project 2									
Project 3									
Project 4									

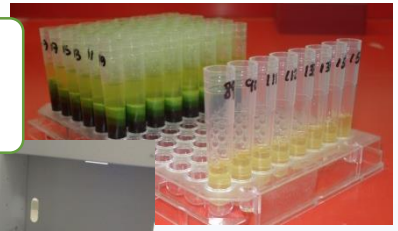
Genotyping order template

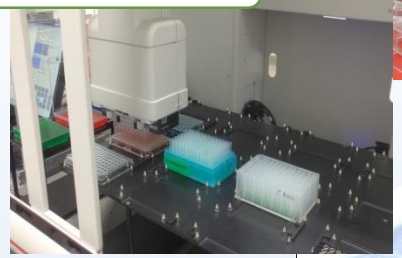


DNA Sample Tracker

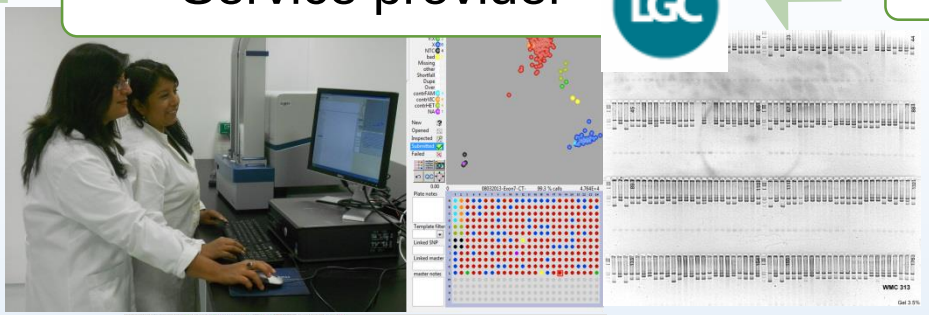
Tissue sampling

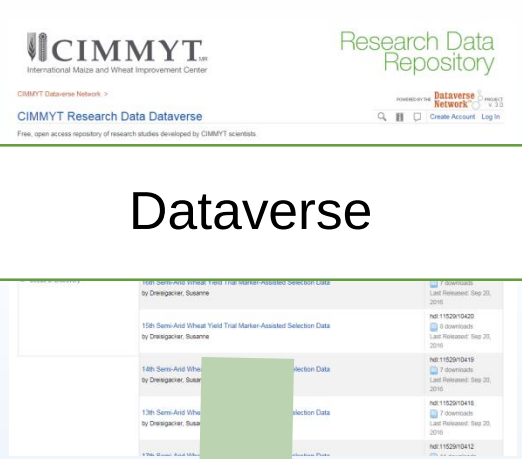
DNA extraction





Genotyping and/or Service provider

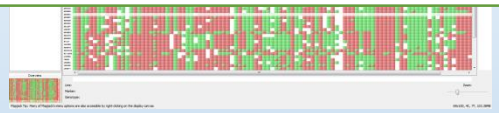




Dataverse

GOBii

Analyses tools



Selection decision

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Current Service providers

- ✓ LGC Genomics, UK: KASP genotyping for MAS/MABC
- ✓ TraitGenetics, Germany: KASP genotyping for trait-based genotyping and marker development
- ✓ KSU, USA: GBS of advanced breeding germplasm
- ✓ SAGA, Mexico: GBS of gene discovery panels and populations
- ✓ TraitGenetics, Germany: SNP genotyping (15K/20K Ilumina and 35K Affymetrix arrays) for gene discovery
- ✓ University of Bristol, UK: SNP genotyping (35K Affymetrix array) for gene discovery
- ✓ Diverse research labs: Exome capture/RNAseq for gene discovery

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Output due to the use of marker technologies

Marker-assisted selected lines in international nurseries

31st Semi-Arid Wheat Yield Screening Nursery

		Disease rating for stem rust							
Selection environment		Total	R	R-MR	MR	MR-MS	MS	MSS	S
Mexico - no MAS	No	159	25	52	30	27	22	3	0
	%		15.7	48.4	67.3	84.3	98.1	100.0	
Mexico - MAS	No	40	23	13	4	0	0	0	0
	%		57.5	90.0	100.0				
Kenya	No	106	44	46	10	5	1	0	0
	%		41.5	84.9	94.3	99.1	100.0		
ALL	No	305	92	111	44	32	23	3	0
	%		30.2	66.6	81.0	91.5	99.0	100.0	

Distribution of marker-selected lines in recent nurseries
30 SAWSN (2012): 22%, 20 SAWYT (2013): 24%
31 SAWSN (2013): 20%, 21 SAWYT (2014): 11%

-> genotypic data of international nurseries are released for further forward breeding

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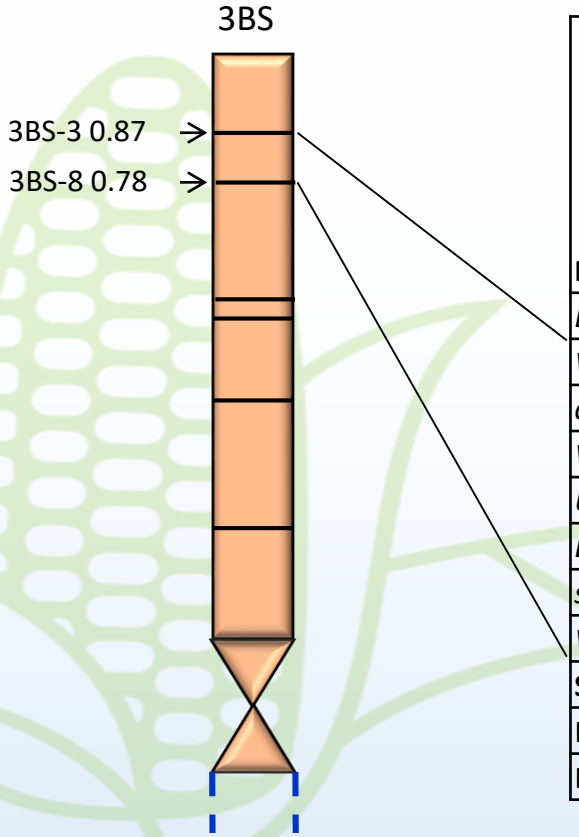
Products due to the use of marker technologies

Genetic resources: Sr2-Fhb1 recombinants introduced into various CIMMYT wheat backgrounds



Marker	Hartog	Sumai3	H-S B/JANZ-Rth5	H-S B/JANZ-Rth5	H-S A/2*MUNAL #1	H-S B/2*NAVJ07	H-S B/2*MUNAL #1	H-S C/2*VORB	H-S C/2*SUP152	H-S D/2*MUNAL #1	H-S D/2*SUP152
Barc102	C	C	D	C	C	C	C	C	C	C	C
WMS389	A	B	A	na	C	C	A	A	A	C	A
csSr2	A	B	A	A	A	A	A	A	A	A	A
WMS533	A	B	B	B	B	B	B	B	B	B	B
UMN10	A	B	B	B	B	B	B	B	B	B	B
Barc133	A	B	B	B	B	B	B	B	B	B	B
snp3BS-8	A	B	B	B	B	B	B	B	B	B	B
WMS493	A	B	C	B	B	B	B	B	B	B	B
Score											
Pbc	1	0	1	1	1	1	1	1	1	1	1
FHB index	7.74	1.18	0.00	0.00	0.00	0.56	4.62	0.00	0.60	0.00	3.97

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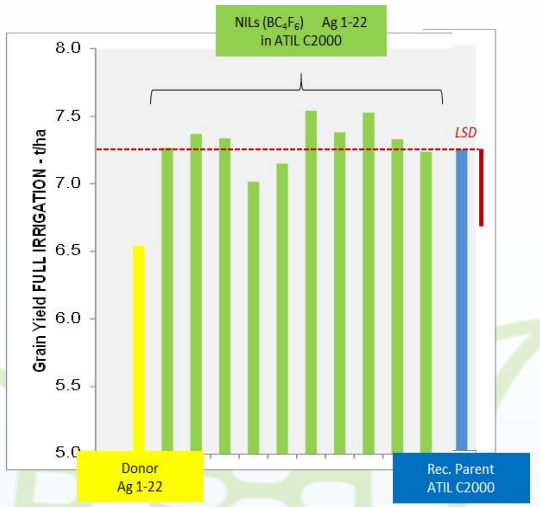
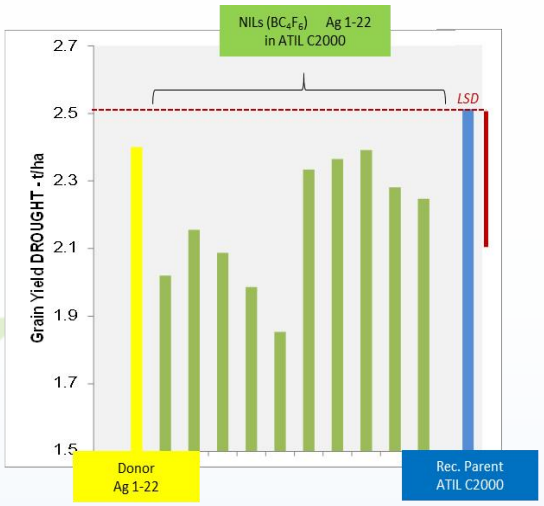
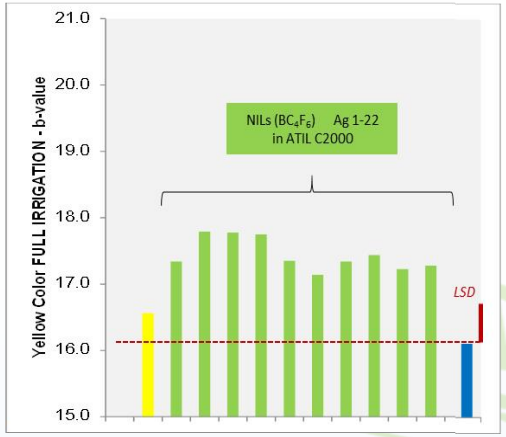
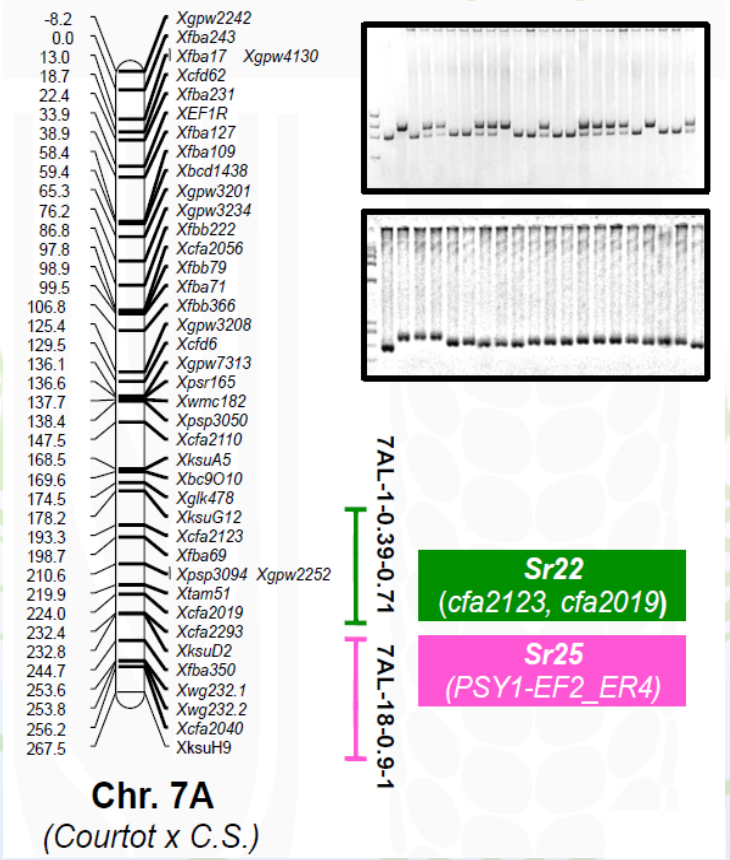
Products due to the use of marker technologies

Genetic resources: new translocations in durum wheat, e.g. L. pontium segment Lr19/Sr25 and Sr22









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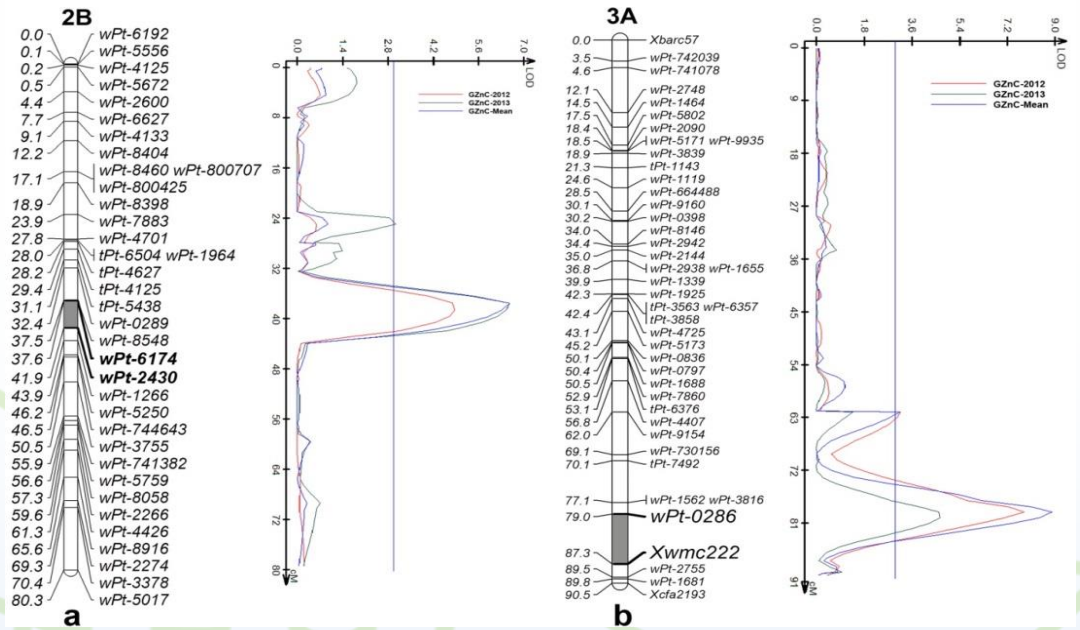
Products due to the use of marker technologies

New QTL/linked markers: Example Zn concentration in wheat grain



						PBW343	Kenya Swara	I-009	I-056	I-021	I-001	I-064	I-149	I-088	I-167	I-124	I-145															
CHR	POS	subject	target	position		3A+, 2B-	3A-, 2B+	QTL for Zinc and Iron, TKW	QTL for Zinc and Iron, TKW	QTL for Zinc and Iron, TKW	QTL for Zinc and Iron, TKW	QTL for Zinc and Iron, TKW	QTL for Zinc and Iron, TKW	QTL for Zinc and Iron, TKW	QTL for Zinc and Iron, TKW	QTL for Zinc and Iron, TKW	QTL for Zinc and Iron, TKW	27 1	27 10	27 11	27 12	27 13	27 14	27 15	27 16	27 17	27 18	27 19						
2B	144.2	2BL	8087406	3174		T	C	C	T	T	T	C	C	T	T	C	C	C	C	C	C	C	C	C	C	C	C						
2B	131.7	2DL	9777308	1312		T	C	C	T	T	T	T	T	C	T	C	failed	C	C	C	C	C	C	C	C	C	C						
2B	129.4	2AL	6401556	17978		T	C	C	C	T	T	C	T	C	C	C	T	C	C	C	C	C	C	C	C	C	C						
2B	78.8	2BL	8089357	8261		A	C	A	A	A	A	A	A	A	A	C	A	ile	A	C	A	A	C	C	A	C	C	ile					
2B	74.9	2BL	8091156	14160		T	C	C	T	T	T	C	C	T	T	C	C	C	C	C	C	C	C	C	C	C	C						
2B	74.1					A	G	G	A	A	A	G	G	A	A	G	G	G	G	G	G	G	G	G	G	G	G						
2B	60.9	2BS	5230118	7003		A	G	A	A	G	G	G	G	A	A	G	G	A	G	A	A	G	A	G	A	G	G						
2B	31.2	2AS	5237970	1084		C	G	G	C	S	G	G	G	C	C	G	G	G	G	G	G	G	G	G	G	G	G						
2B	27.3	2BS	5240221	12736		A	G	G	A	G	G	G	G	A	G	A	A	G	A	G	G	A	G	A	G	ile	A	A					
3A	51	3AS	2602511	1658		C	T	C	C	T	C	C	Y	C	T	T	C	T	T	T	C	T	C	C	C	T	C	C					
3A	52.5	3AS	3370051	4831		A	G	A	A	G	A	A	R	A	G	G	A	G	G	G	A	G	A	A	A	G	A	A					
3A	53.3	3AS	3414620	5835		T	C	T	T	C	T	T	Y	T	C	C	Y	C	C	C	C	C	C	C	C	C	C	C					
3A	56.8	3AL	4369382	8385		C	T	C	C	T	C	C	Y	C	T	T	Y	T	T	T	T	T	T	T	T	T	T	T					
3A	61.4	3AL	4292425	1242		C	T	C	C	T	C	T	C	C	T	C	failed	T	T	T	T	T	T	T	T	T	T	T					
3A	62.9	3AL	4330910	2758		A	G	G	A	A	A	G	G	A	G	G	G	A	A	A	A	A	A	A	A	A	A	A					
3A	69.2	3AL	4404424	3976		C	T	T	C	C	C	T	C	C	T	C	C	Y	C	Y	T	C	T	Y	T	C	Y	C					
3A	81.6	3AL	3396460	540		G	T	T	G	G	G	T	T	G	T	T	G	G	ile	G	K	ile	K	T	K	ile	T	ile					

Reference: Hao et al. (2014)

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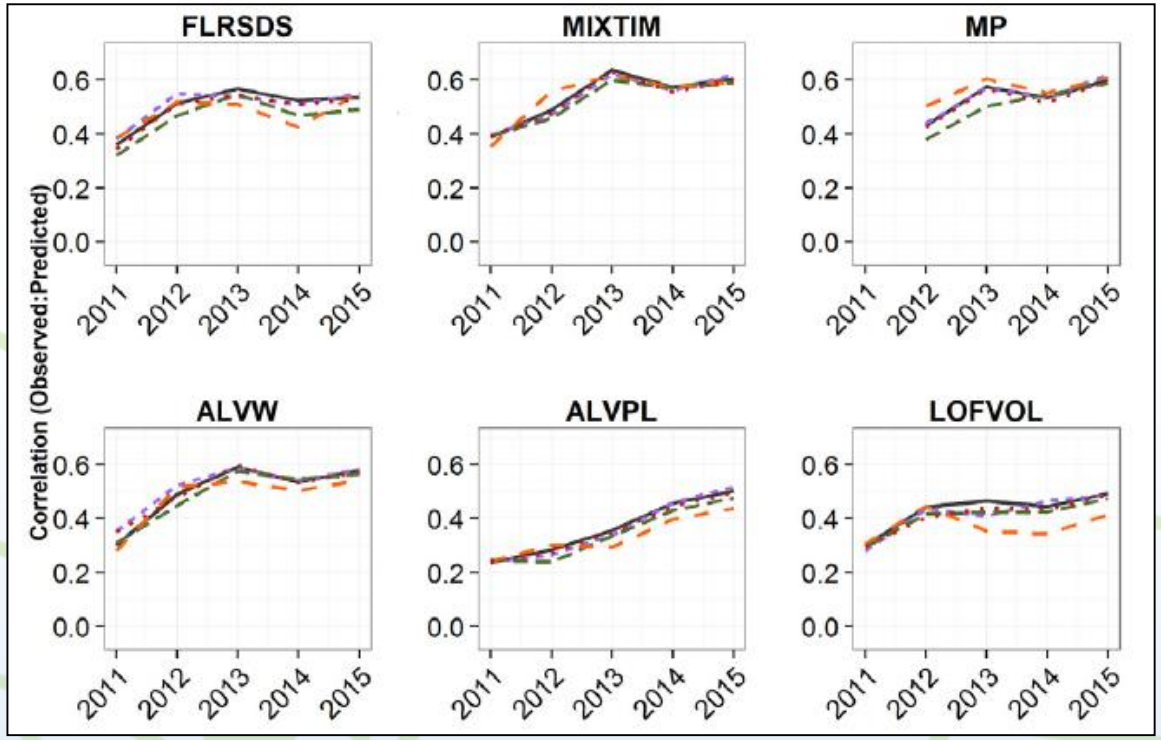
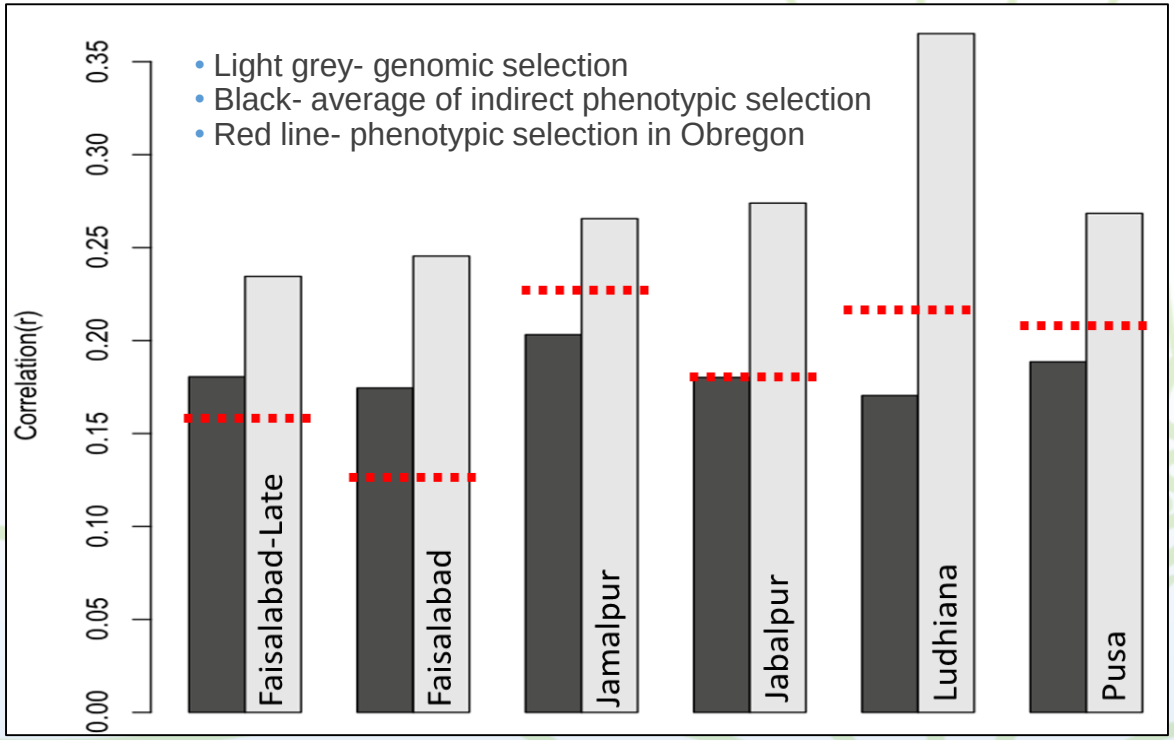
Products due to the use of marker technologies

Genomic predictions applied for quality analyses and multiple environments

Prediction accuracy for different quality traits:



Prediction accuracy across multiple environments in India



References: Battenfield et. al 2016, Guzman et. al 2016, Rutkoski et. al 2016

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HTPG project needs vs. expectation

Leaf tissue sampling

- *Concern about the throughput/functionality of the EP100*
- *Scissors not optimal*

Option to send both DNA and/or tissue samples to Intertek

- *Use the same DNA needs to be used to add STS marker genotyping*

Flexible and larger sets of SNPs

- *Set of > 30 SNPs for trait-based genotyping*
- *Set of genome-wide SNPs of MABC background selection*

Turn-around time

- *Turn-around time needs to be guarantied*

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Communication with other CG centers and end users

CIMMYT/ICARDA: One Wheat Program

> CIMMYT will provide access to sequencing, molecular characterization capacity

CIMMYT-IWYP hub:

> Introgression of yield potential traits developed by advanced research institutions

INIFAP – National Program in Mexico

> Service user through CIMMYT

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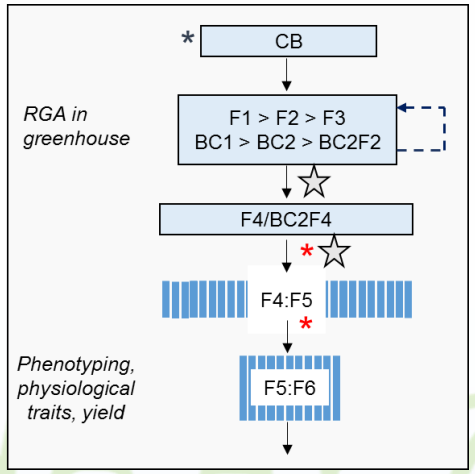
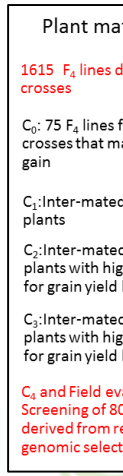
New and upcoming initiatives in the GWP

- Greenhouse set-up for RGA





- Forward breeding for Fusarium head blight resistance, nutritional quality, soft durum wheat, APR leaf rust resistance in durum wheat

- Incorporate trait-based genotypes in genomic prediction models (IWYP-hub, hybrid wheat)

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Application and Adoption

- SNP sets
  - Set 1: Disease resistance genes (Rust, Fhb, Nematodes)
  - Set 2: Phenology (Vrn, Ppd, Eps)
  - Set 3 is to come: Durum wheat (Rust, quality, Nematodes)

- Validation set (Jan)
  - Test AK-EP-100 in the greenhouse (arrival date?)

- Germplasm
  - Crossing block BW for forward breeding (Jan-Feb)
  - International nurseries for trait based genotyping (Jan-Feb, Jun)
  - BC1 for MABC within IWYP (Feb)
  - BC2 for MABC within BW (Mar)
  - GS panel within IWYP (Mar)

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Additional aspects

- SNP data return
  - Data format
  - Ability of SNP re-calling
- IP
  - Agreement between Intertek and LGC
  - Agreement between Intertek and CG centers
- GBS/RepGen across CG centers
  - Initial RepGen testing with SAGA for wheat

Summary

- Development of marker-assisted selection strategies has been initiated in the different CIMMYT wheat breeding programs
- Increasing implementation of forward breeding strategies is required
- Large number of trait-linked KASPs are available but not necessarily for the ‘must have’ gens of forward breeding gene targets.
- For some traits SSR/STS markers need to be converted to SNPs to fully exploit the Intertek platform e.g. FHB, STB, Nematodes
- Target of 150K is still a challenge
- Logistics and turn-around time will be key factors for success.

Thank you for your attention