Kompetetive Allele Specific PCR KASP markers
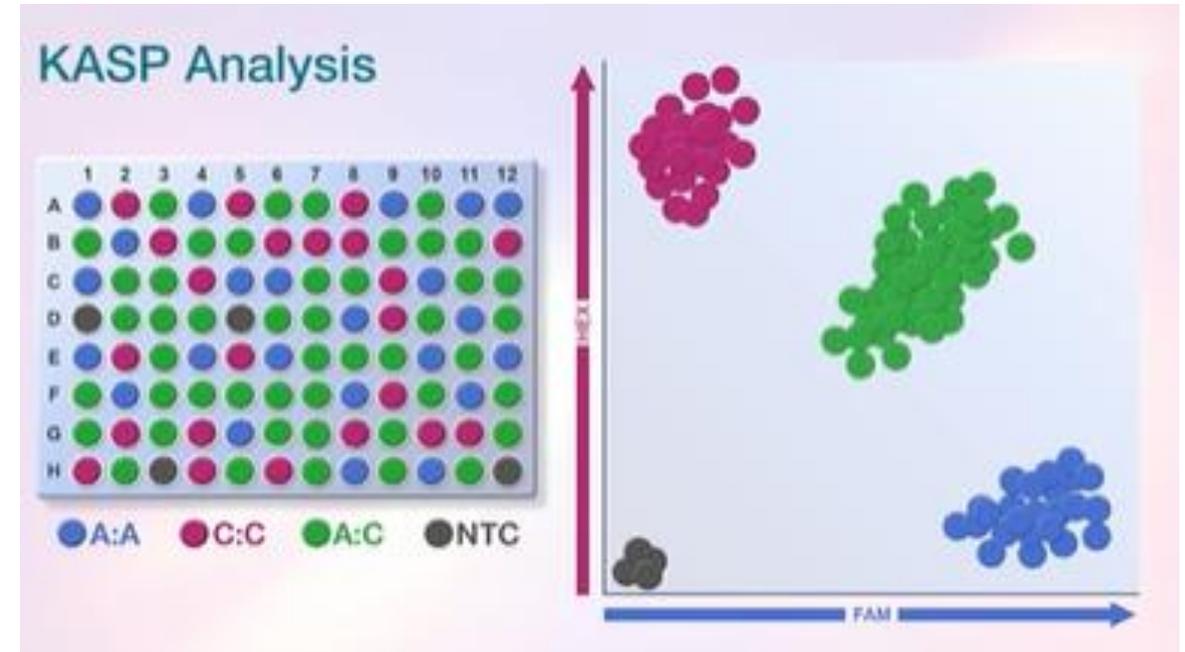
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CIMMYT

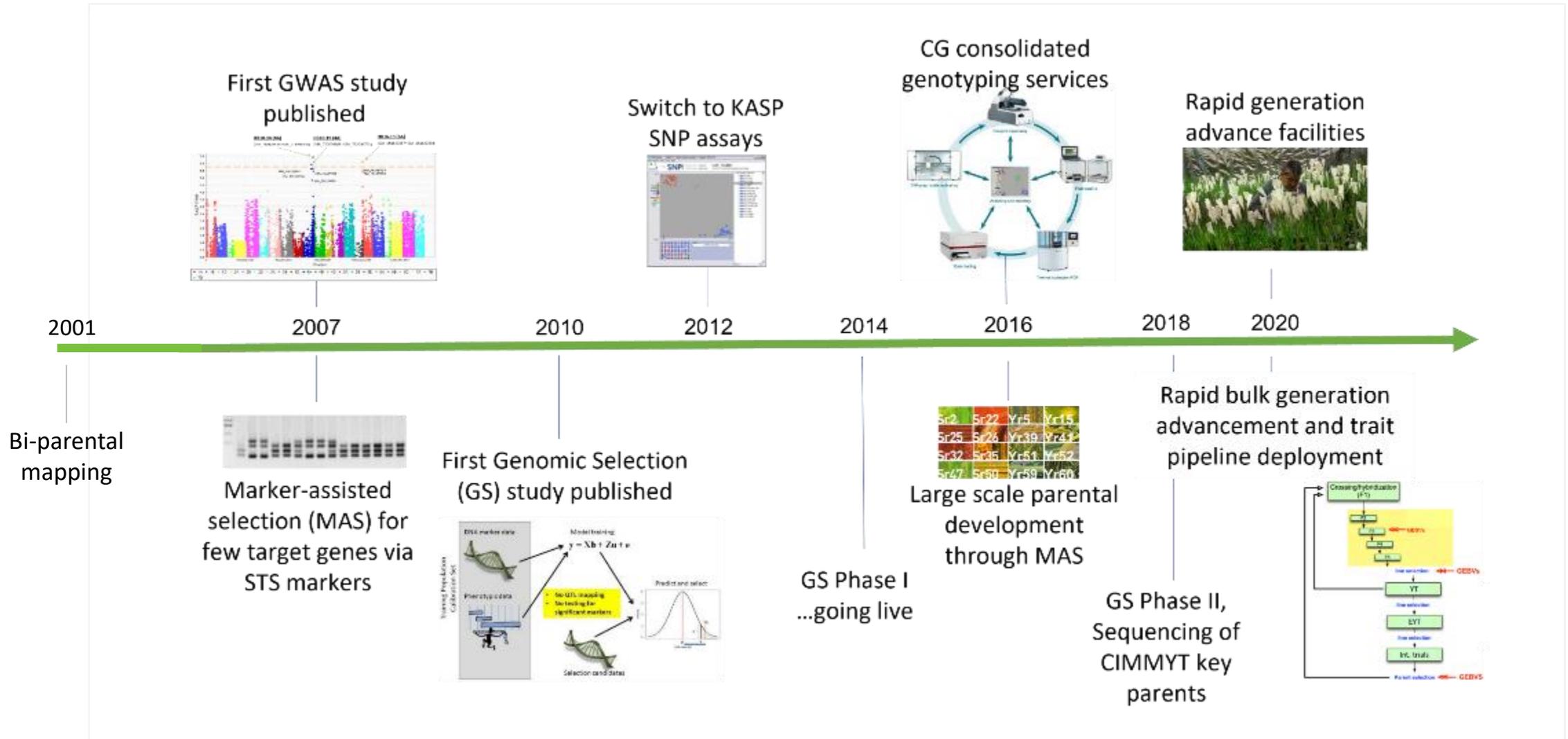# Kompetetive Allele Specific PCR: KASP

Monoplex SNP platform, supports mainly low- to mid-density SNP applications, e.g., MAS, MABC, parental characterization, QC

LGC Biosearch Technologies
https://www.biosearchtech.com

Biallelic characterization of SNPs and Indels

Integration of genomics tools in the CIMMYT GWP
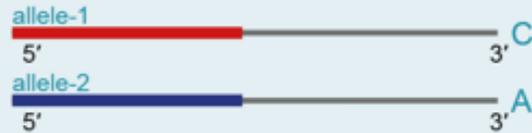
How does KASP work ?

1. Assay components:

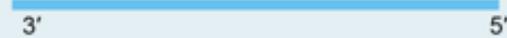**A) KASP Assay Mix**: consists of 2 allele specific primers and 1 reverse primer.

B) KASP Master Mix: contains universal fluorescent probes, Taq polymerase and dNTP's in an optimised buffer solution.
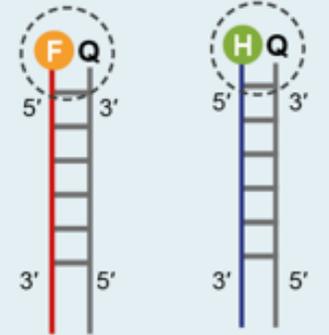
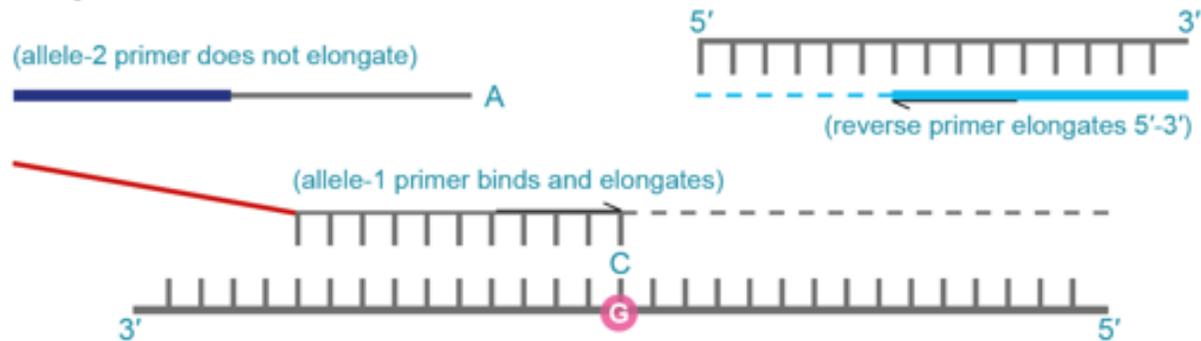### A) KASP Assay Mix

Allele-specific forward primers:

allele-1
5' ———————— 3' C

allele-2
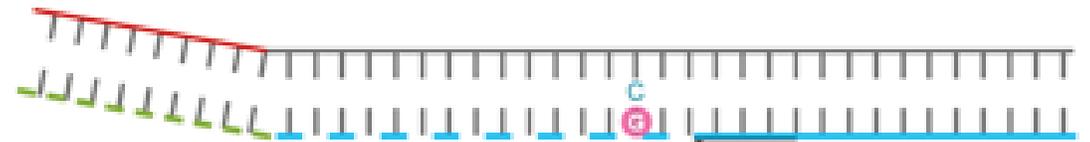5' ———————— 3' A

Reverse primer:
3' ———————— 5'

B) KASP Master Mix

2. Denatured template and annealing components – PCR round 1:

(allele-2 primer does not elongate) — A

(reverse primer elongates 5'-3')
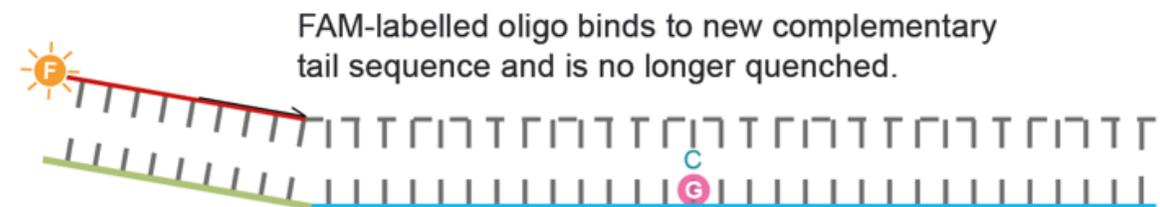
(allele-1 primer binds and elongates)

C

3' — G — 5'

In the first round of PCR, one of the allele-specific primers matches the target SNP and with the common reverse primer, amplifies the target region.

## 3. Complement of allele-specific tail sequence generated – PCR round 2:

C
G

4. Signal generation – PCR round 3:

FAM-labelled oligo binds to new complementary tail sequence and is no longer quenched.

C
G

KASP assay - advantages

Easy to use

❖ Requires only two components: KASP assay and master mix

❖ Compatible with relative crude DNA extraction methods

❖ Flexible primer design

❖ Signal can be read on qPCR and FRET-capable plate readers

❖ Scaling up is possible
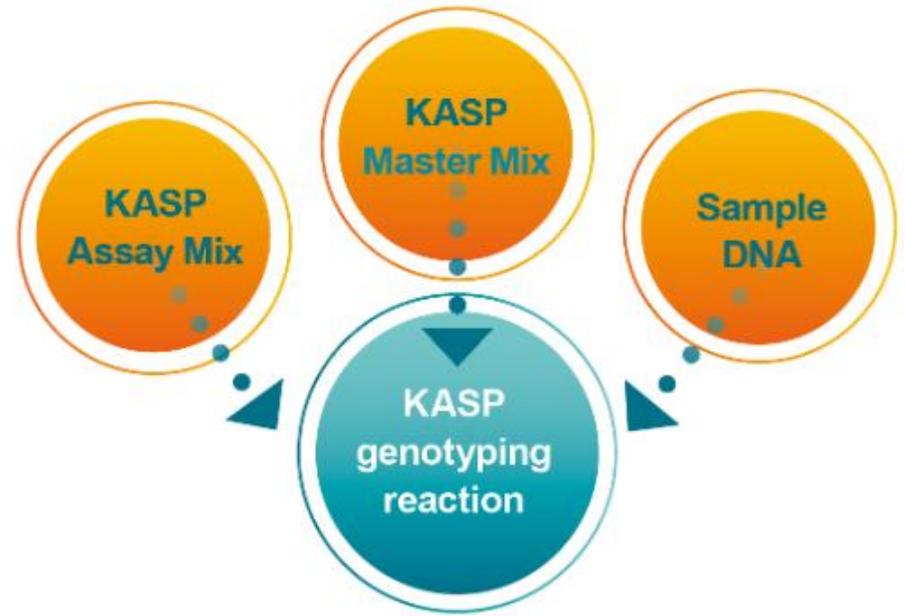
Low cost

❖ Low reaction volumes – down to 1 uL - keep reagent costs to a minimum

❖ Relative robust assay means less time spent on repeats

❖ Cost benefits enable you to perform more assays overall, improving the quality of

KASP assay - disadvantages

❖ Less competitive for medium- and high-density SNP applications

❖ To expensive compared to upcoming medium-density platforms when > 100 SNPs required.

❖ Cost- volume ration of most service providers

KASP genotyping in the CIMMYT GWP

Wheat Molecular Breeding Lab (WMBL)

- KASP design, initial verification and validation
- Small projects
- Complex production markers (more flexible protocols)
- (Gel-based markers)

EIB Low density genotyping services (LDSG)

- Fully optimized production markers
- Fast turn-around (some recent tissues with customs release with longer turn-around)
- Min. or multiples of 384 samples

KASP marker design – PolyMarker (http://www.polymarker.info/)

PolyMarker: bioinformatics pipeline for SNP assay development, via alignment to reference genomes


```
>Ta#S58861868:A214G-Parent1        CATACTGATGACACGATTGGCTACSGGCCTTGAAGATAGMAGCAGATAACTTCAGTGTAATCCAAGTTGACTG
>Ta#S58861868:A214G-Parent2        CATACTGATGACACGATTGGCTACSGGCCTTGAGGATAGMAGCAGATGACTTCAGTGTAATCCAAGTTGACTG

>iwgsc_css_1AS_scaff_3271989        CATACTGATGACACGATTGGCTACGGGCCTTGAGGATAGCAGCAGATAACTTCAGTGTAATCCAAGTTGACTG
>iwgsc_css_1BS_scaff_3474683        CATACTGATGACACGATTGGCTACCGGCCTTGAAGATAGAAGCAGATAACTTCAGTGTAATCCAAGTTGACTG
>iwgsc_css_1DS_scaff_1913057        CATACTGATGACACGATTGGCTACCGGCCTTGAGGATAGCAGCAGATAACTTCAGTGTAATCCAAGTTGACTG

>MASK                               ------------------------G--------:------C-------&------------------------
```

- Use available genomics resources to support your SNP design e.g., GrainGenes, EnsemblPlants, etc,..

- Main problem in wheat is its polyploidy

- KASP Assay Cost: 40-45 USD (T4OLIGO, 100 μM, local provider)

- Other primer design software can be used, e.g., Primer3, WASP

KASP genotyping

PCR

- 384-well plate set-up for PCR

- KASP Master Mix: PACE™ Genotyping Master Mix, 3PC Bioscience: 4,920 USD (250ml)

- 4.25 µl – PCR reactions

Data visualization

- Initially qPCR, excel

- PHERAstar FSX plate reader, KlusterCaller

https://repository.cimmyt.org/handle/10883/18822

KASP genotyping in the CIMMYT GWP

Rust resistance in bread wheat

Resistance genes

Sr2 Sr22
Sr25 Sr26
Sr32 Sr35
Sr47 Sr50

Yr5 Yr15
Yr39 Yr41
Yr51 Yr52
Yr59 Yr60

Lines in current yield trials

Cross	Gene	# lines YT18-19
BOKOTA*3//RL6077/AOC-YR	Lr67	1
BORL14*3/SUJATA	Lr67 + QTL.7BL	1
HARTOG_SUMAI3 (LINE B)/2*NAVJ07/5/KIRITATI//HUW234+LR34/PRINIA/3/FRANCOLIN #1/4/BAJ #1/6/2*TAM200/PASTOR//TOBA97/3/FRNCLN/4/WHEAR//2*PRL/2*PASTOR	Sr2 + Fhb1	2
HARTOG_SUMAI3 (LINE B)/2*NAVJ07/4/MUTUS//KIRITATI/2*TRCH/3/WHEAR/KRONSTAD F2004/5/2*TAM200/PASTOR//TOBA97/3/FRNCLN/4/WHEAR//2*PRL/2*PASTOR	Sr2 + Fhb1	1
SWSR22T.B.//TACUPETO F2001*2/BRAMBLING/3/2*TACUPETO F2001*2/BRAMBLING/4/MUNAL #1/5/2*MUNAL*2/WESTONIA	Sr22	1
SWSR22T.B.//TACUPETO F2001*2/BRAMBLING/3/2*TACUPETO F2001*2/BRAMBLING/4/SUP152/BAJ #1/5/2*KUTZ	Sr22	1
SWSR22T.B./FRANCOLIN #1//2*FRNCLN/3/3*WBLL1*2/BRAMBLING*2//BAVIS	Sr22	2
MUTUS*2/HARIL #1*2/3/SWSR22T.B./2*BLOUK #1//WBLL1*2/KURUKU/4/MUCUY	Sr22	4
SWSR22T.B.//TACUPETO F2001*2/BRAMBLING/3/2*TACUPETO F2001*2/BRAMBLING/4/3*KACHU/3/WHEAR//2*PRL/2*PASTOR	Sr22 + Sr25	2
BORL14*3/4/KACHU/3/WHEAR//2*PRL/2*PASTOR	Sr25	3
SHORTENED SR26 TRANSLOCATION/4/3*CHIBIA//PRLII/CM65531/3/MISR 2/5/2*BAJ #1	Sr26	1
SHORTENED SR26 TRANSLOCATION//2*WBLL1*2/KKTS/3/BECARD/4/3*BORL14	Sr26	1
FRANCOLIN #1*2//ND643/2*WBLL1/3/SWSR22T.B./2*BLOUK #1//WBLL1*2/KURUKU/5/SHORTENED SR26 TRANSLOCATION/4/3*CHIBIA//PRLII/CM65531/3/MISR 2/6/SWSR22T.B./2*BLOUK #1//WBLL1*2/KURUKU	Sr26 + Sr22	4
BORL14*2/SR50	Sr50	7
BORL14*3/SR50	Sr50	7
BORL14*3/BLANCA GRANDE 515	Yr5 + Yr15	13
BORL14*2/BLANCA GRANDE 515	Yr5 + Yr15	6
BLANCA GRANDE 515/2*BAJ #1	Yr5 + Yr15	4
BORL14*3/KOELZ W 11192:AE	Yr52	3
YR57#5474-6/3*BAJ #1	Yr57	2
BORL14*3/IRAGI	Yr59	2
BORL14*2/IRAGI	Yr59	1
BORL14*3//LALBMONO1*4/PVN	Yr60	3
BORL14*2//LALBMONO1*4/PVN	Yr60	1
LALBMONO1*4/PVN//3*BAJ #1	Yr60	1

Combining genes for fusarium head blight resistance

Sr2-Fhb1 recombinants introgressed in CIMMYT elite backgrounds
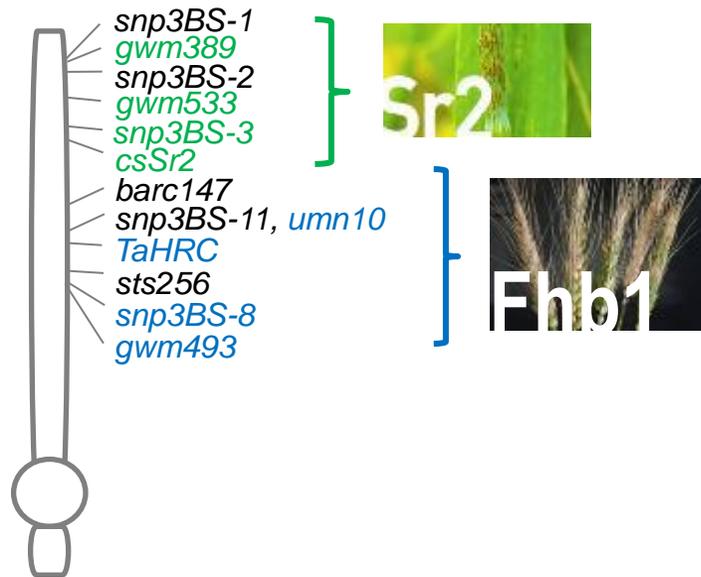
Pyramiding of complementary genes
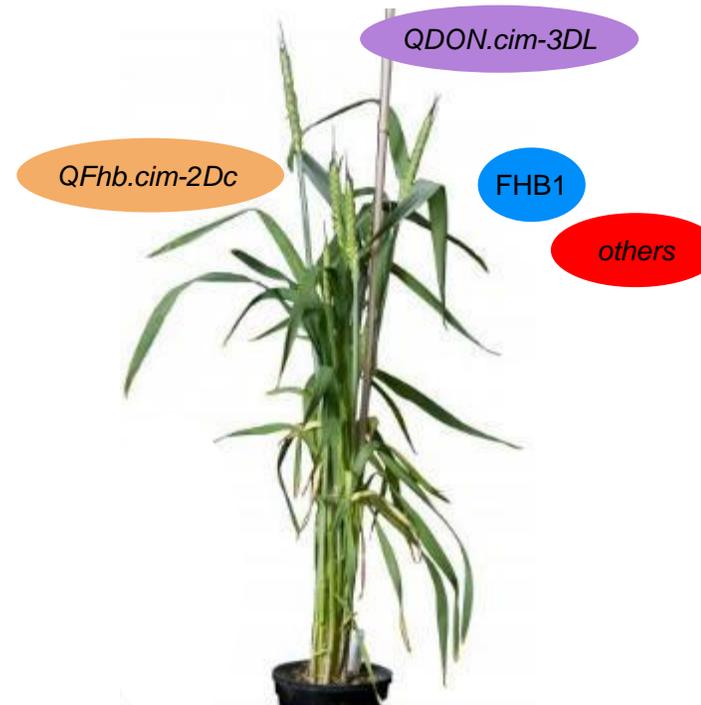
Centralized marker-assisted trait development pipelines

Parent development pipeline (Trait deployment)	**Trait augmentation pipeline** (Trait augmentation)	**Discovery pipeline** (Trait Discovery)
Parent development by embedding or new genetic variants in diverse genetic backgrounds	Frequency augmentation of relevant genetic variants, final product development	Identification of new diversity, trait donors and genetic variants

Previous parent development pipelines based on the shuttle through DGGW, IWYP, SEED, CRP and other projects

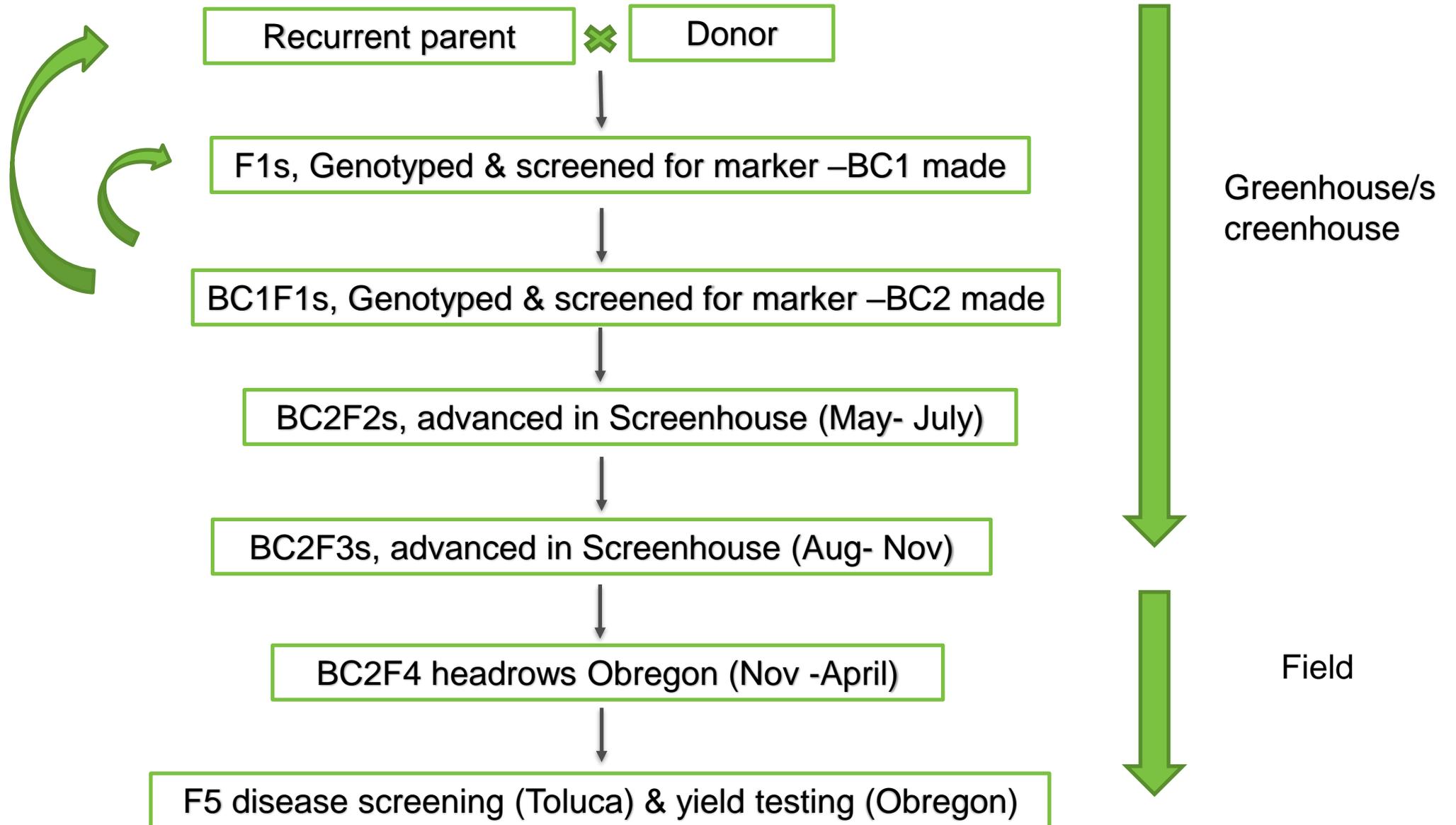CGIAR   CIMMYT

Wheat breeding scheme optimization in Toluca

Trait augmentation pipelines: priorities 2020 - 2022

Crop	Project Value	Product Profile	Trait	Genes
BW	Improved and diversified rust resistance	HW-OE-NM, HW-HT-EM, HW-DT-NM, HW-DT-EM	Stem and yellow rust	*Sr22, Sr50, Sr2, Yr57, Yr59, Sr35, Yr15, Yr5, Sr47, Sr25, Sr13, YrSP*
BW	Enhanced Fhb resistance	HW-HiR_NM	Fusarium head blight	*Fhb1, Qfhb.cim-2DLc*
BW	Improved STB resistance	HW-HiR_NM, HW-DT-NM	Septoria tritici Blotch	*Stb6, Stb16*
BW	Improved insect resistance	HW-OE-NM, HW-DT-NM	Green bug	*Gb7/Gba, Gb5, QRp.slu-5AL, QRp.slu-5BL-R*
BW	Improved TKW	HW-OE-NM, HW-HT-EM, HW-DT-NM, HW-DT-EM	TKW	*TaGW2, Qgw-jic-6A, Qgl-jic-5A*
BW	Novel diversity for stress tolerance (heat drought)	HW-DT-EM, HW-DT-NM, HW-HT-EM	Heat/drought tolerance	*LTP-HB3B, LTP-HB4B, LTP-HB5B, LTP-HB6D, LTP-HB7D-1, LTP-HB7D-2*

Speed breeding for rapid introgressions of specific genes

Thank you for your interest!