Implementing new technologies for effective MAS to enhance rice breeding

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IRRI CGIAR

➢ Focus: Application of MAS and pre-breeding products
- OneRice breeding strategy
- Applications of MAS in breeding

➢ Application of MAS
- Genotyping platforms
  → Mid-density (RiCA, skim-sequencing)
  → Major-gene selection
- Cost comparison
- Choosing major genes
- What to select, when
- Disease resistance stewardship

➢ Opportunities for MAS in rice
- NTDD products
- Blast resistance

Novelty ≠ Effectiveness
Technology ≠ Cost

Focus on technologies that are:
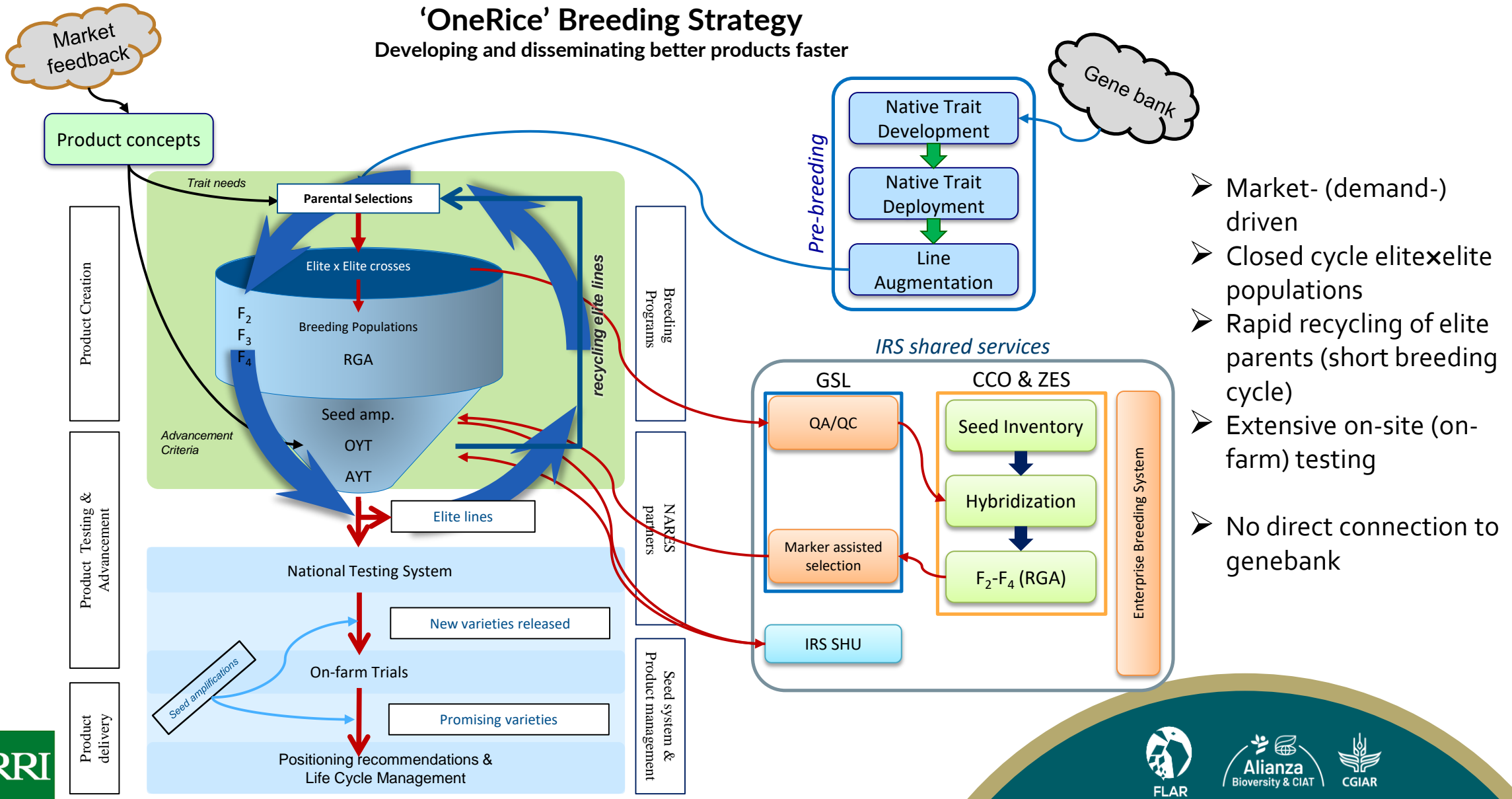➢ Effective
➢ Affordable

OneRice breeding strategy

Applications of MAS in breeding
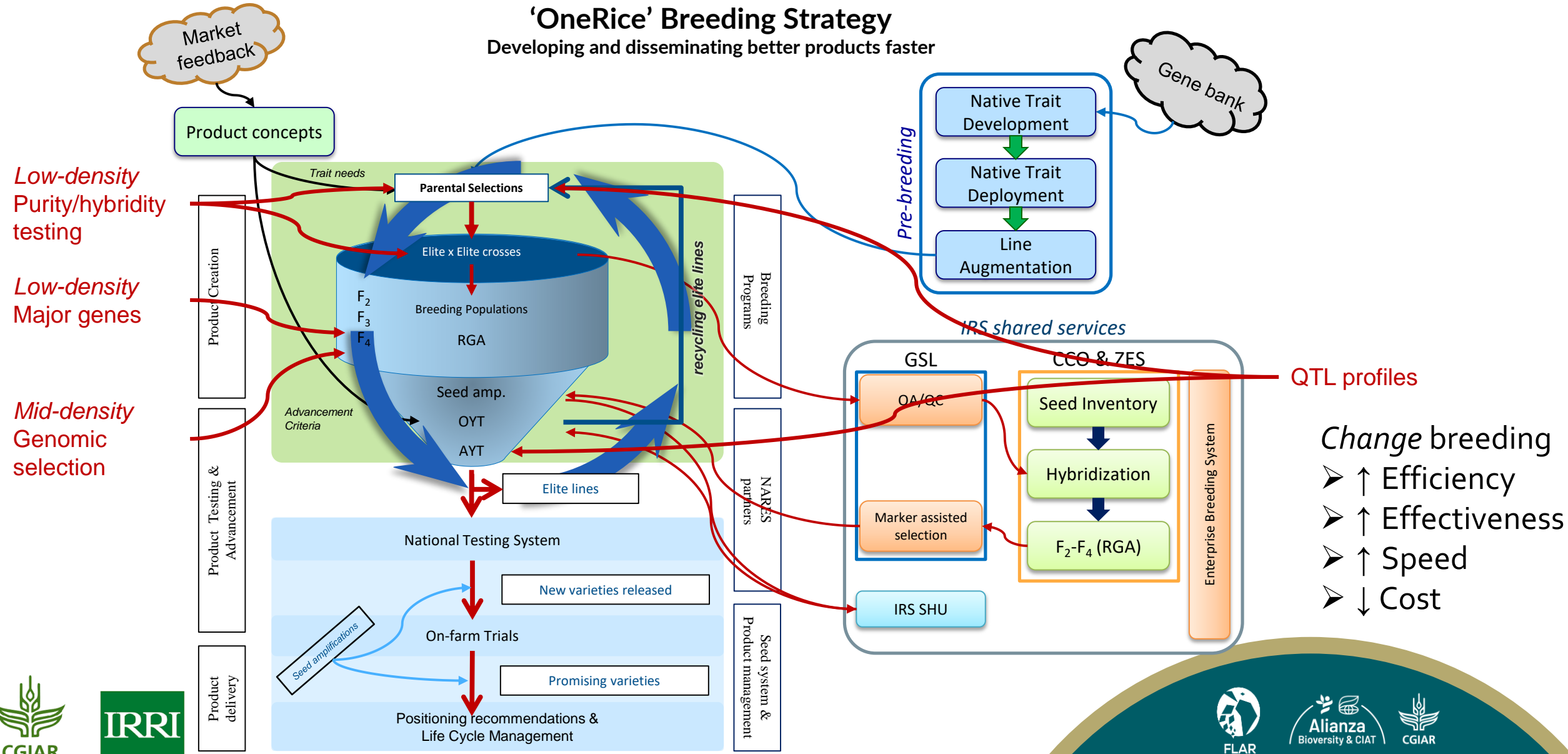
Application	Technology	Density	Sample throughput	Platform	Price
Hybridity/purity	KASP	Low	High	**Intertek**	$3/sample
Major-gene selection	KASP	Low	Very high	**Intertek**	$3/sample
Genomic selection, variety fingerprinting	Amplicon sequencing	Medium	Moderate	**Agriplex DArTag**	$13/sample
Deployment	KASP	Low	High	In-house	$3/sample
Augmentation	KASP	Low	High	**Intertek**	$3/sample
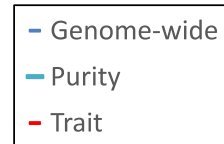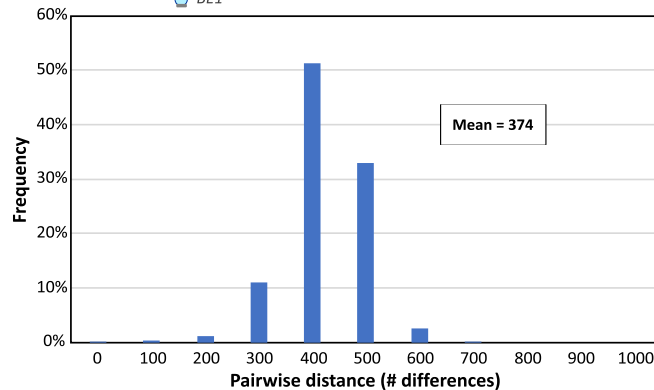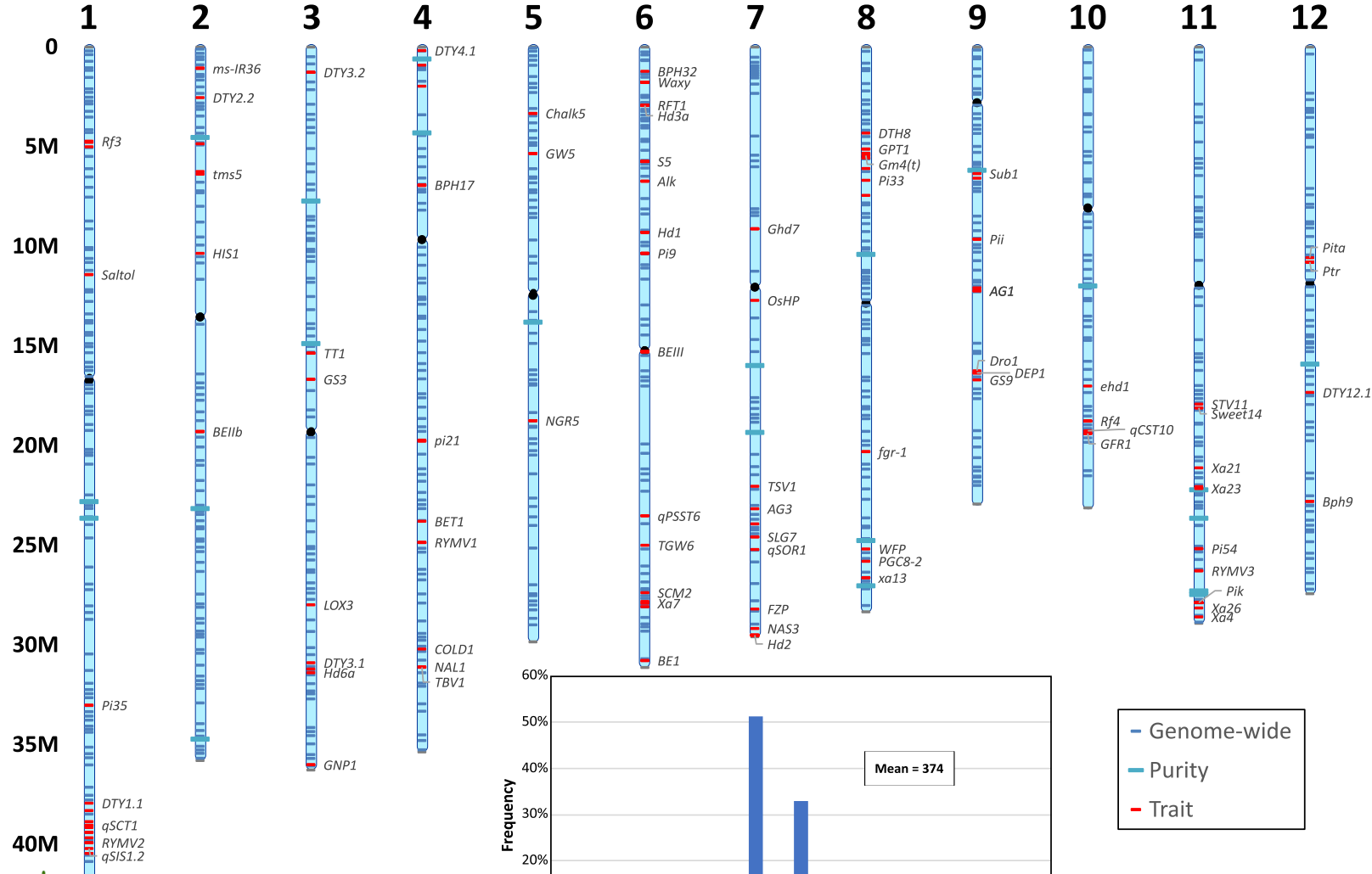
RiCA v4 - Agriplex

Mid-density panels

Purpose:
➢ Enable genomic selection
➢ Major-gene fingerprinting (QTL profiling)

Implementation:
➢ 1k RiCA v4 panels @ Agriplex, DArT $13/sample
➢ All material under seed amplification genotyped after MAFB selections
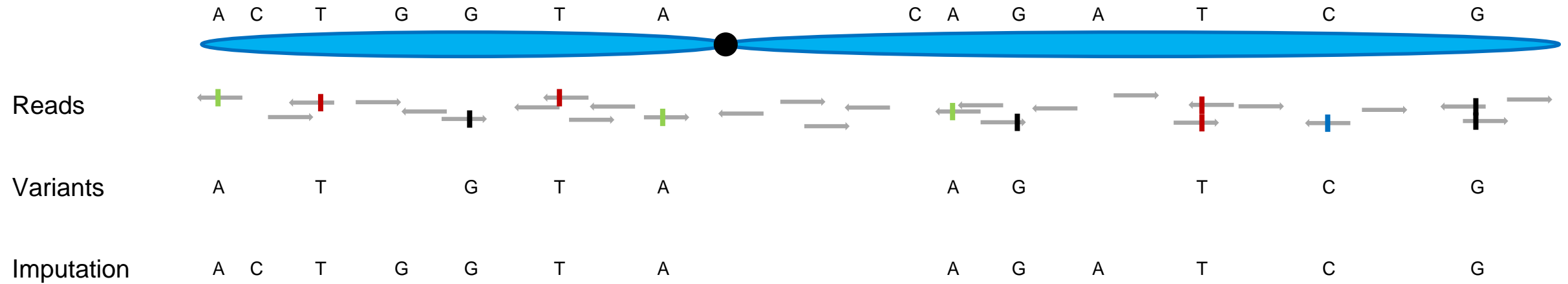
Advantages
- ➢ High density
- ➢ *Germplasm-agnostic*
  - • Same platform and analytics pipeline irrespective of germplasm
- ➢ Well connected to historical datasets (imputation)
- ➢ Cost-parity

Disadvantages
- ➢ Less certain about QTL profiling
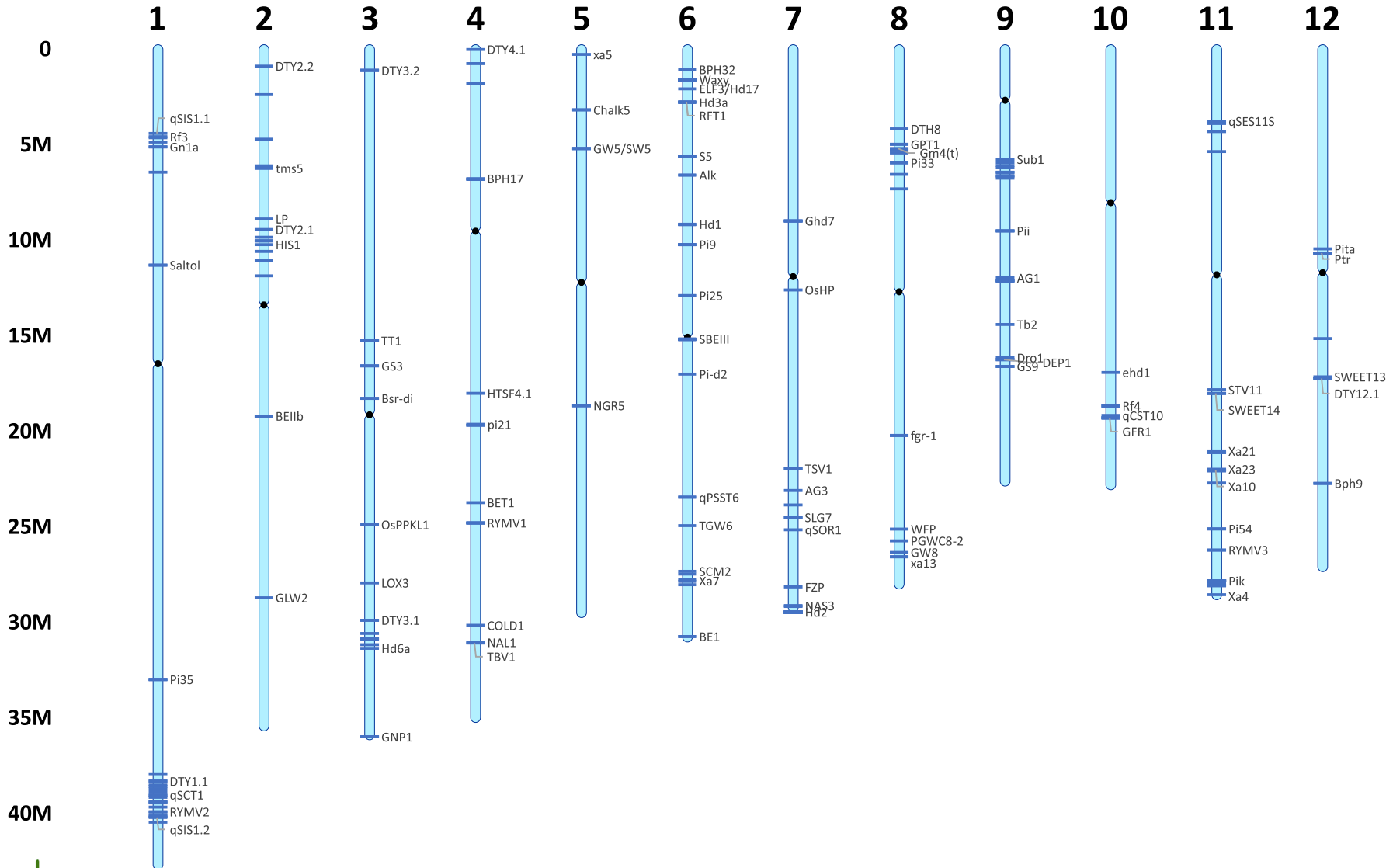
Intertek panels

➤ High throughput, low-cost marker systems implemented

➤ Low density, customized genotyping

➤ >90 major genes/QTLs represented

➤ Accurate across all elite material: confident selection

➤ Publicly accessible on **Intertek** platform and mid-density panels

https://www.intertek.com/agriculture/agritech/
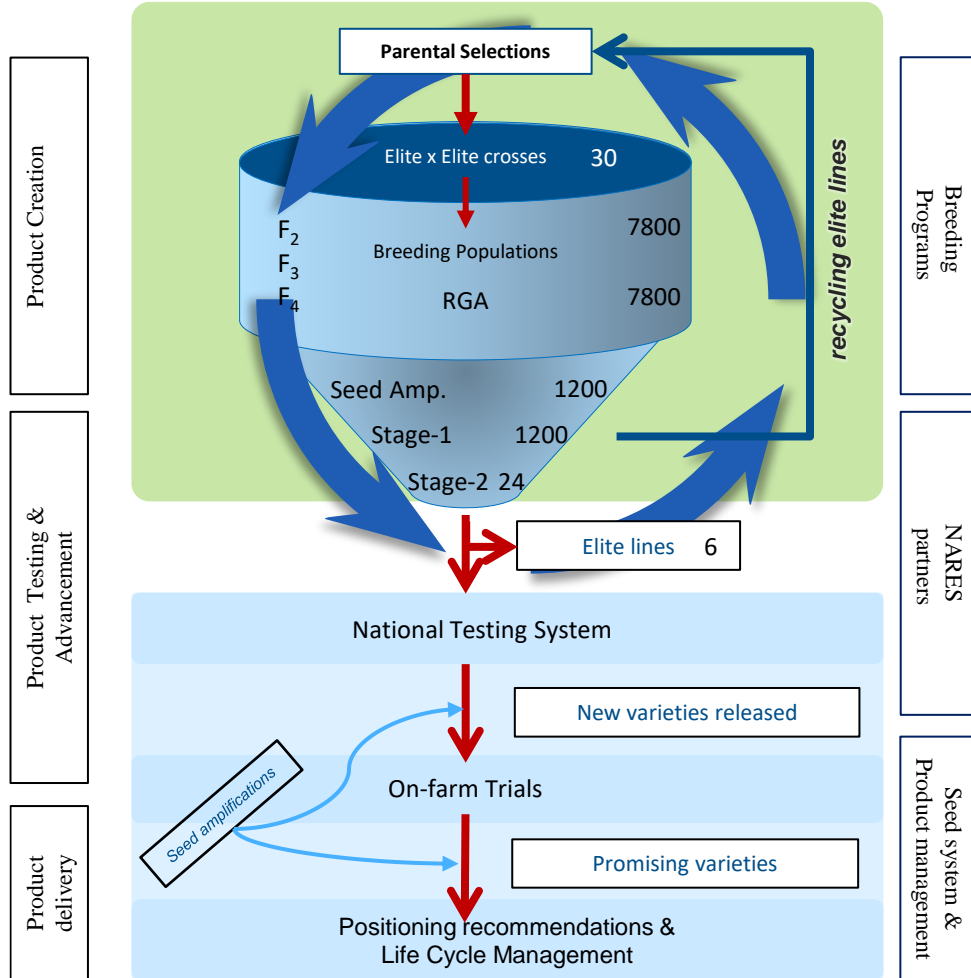
	Without MAS	With MAS
MAS (major-gene)		7800 lines x $2.50 = $19,500
Mid-density (Genomic prediction)	7800 lines x $13 = $101,400	1200 lines x $13 = $15,600
Field trialing	7800 lines x 1.5 sites/line x ($5000/site, 300 lines) = $195,000	1200 lines x 1.5 sites/line x ($5000/site, 300 lines) = $30,000

F=5%, R=20	1-stage selection	2-stage selection	
Q (# Loci)	Fixation at F4	F2 enrichment, fixing 1 gene	F4 fixation, 1 gene fixed
1	60	107	
2	142	85	43
3	329	90	77
4	757	106	137
5	1738	131	237

- For the same population size, MAS is much cheaper
- For a given program size, MAS produces higher quality segregants

Choosing major gene targets
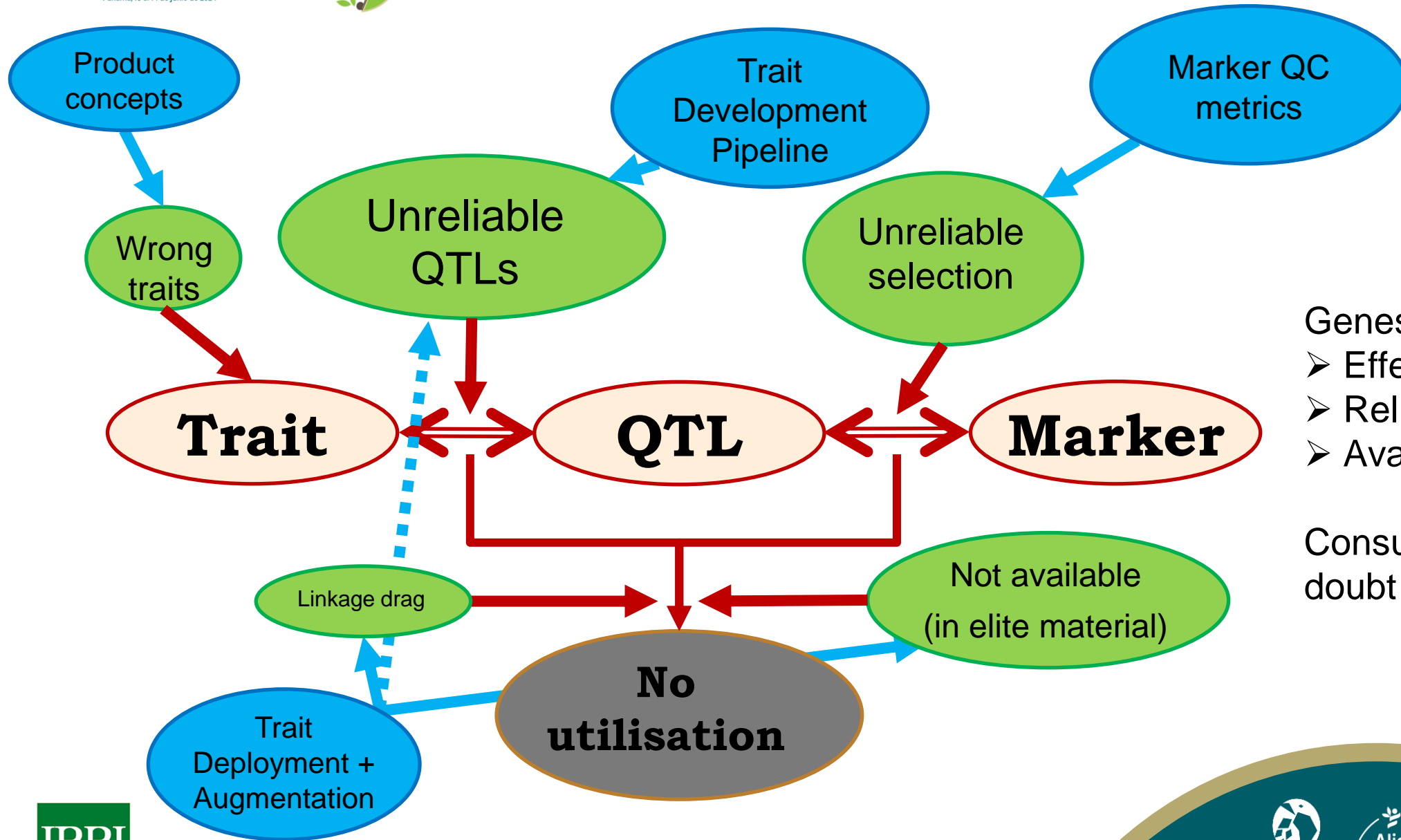
Genes must be:
➤ Effective (for trait)
➤ Reliable markers
➤ Available in elite

Consult experts if in doubt

| Trait importance	Within-stage priority	Markers	Markers	Seed Amplification	Stage 1	Stage 2	Stage 2
		Strict selection in RGA *Rare in elite material	Opportunistic selection in GS * Common in elite programs	* 1000 lines/1x program	Genomic selection * 1000 lines/1x program	Phenotypic confirmation (marker effects plus Stage 1)	Low-throughput screening
Game changer	1	RYMV			Head rice recovery	RYMV	Head rice recovery
	2						False smut
	3						
Must have	1	Brown planthopper	Chalkiness	Maturity	Soft eating quality	Chalkiness	Soft eating quality
	2	Blast - neck	Amylose		Lodging	Amylose	Lodging
	3	Blast - leaf	BLB		Brown planthopper	Brown planthopper	
	4	BLB	Maturity		Heat- Reproductive	Blast - neck	
	5					Blast - leaf	
	6					BLB	
	7						
Nice to have	1	Aroma	Tungro		Early vigour	Aroma	Early vigour
	2	Salinity- Vegetative			Early emergence	Salinity- Vegetative	Early emergence
	3	Grain Zn (Med-High)			Grain Zn (Med-High)	Grain Zn (Med-High)	Root Knot Nematode
	4	Drought- Reproductive			Root Knot Nematode	Drought- Reproductive	Brown spot
	5	Tungro			Salinity- Reproductive	Tungro	Sheath blight
	6	Submergence			Grain Fe (Med+)	Submergence	Sheath rot
	7	Anaerobic germination			Grain protein	Anaerobic germination	Stagnant flooding
	8				Cold- Reproductive		Bacterial leaf streak
	9				Drought- vegetative		
	10				Stagnant flooding		
	11				Cold- vegetative		
	12						

➢ Numerous opportunities for MAS

➢ Need to prioritise: 2-3 genes per cross

➢ Selection "index" for major genes (separate from index for polygenic traits) – special consideration for disease resistance

➢ *Consistent:* Increase frequency over breeding cycles, eventually achieving fixation
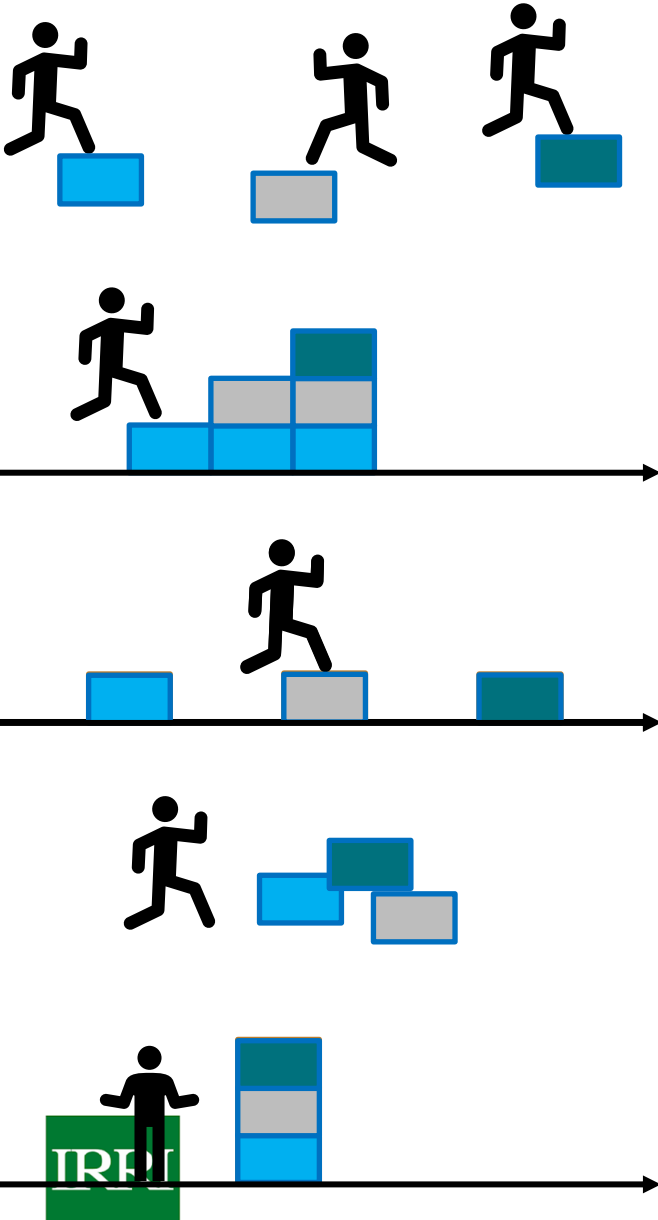
Random deployment (<u>current situation</u>)
- Rapid breakdown
- Poor stewardship

Pathogen

Resistance gene

Sequential deployment
- Sequential short term resistance
- Long-term: sequential breakdown: Very bad

Alternating or targeted deployment
- Deploy new genes singly, temporally or regionally
- Possible but requires strong centralised control; untenable for rice at this time

Variety mixtures
- Promote durability by reducing pathogen population
- However still only requires one mutation to overcome

Pyramid deployment
- Robust resistance, validated in many species
- Requires adherence to pyramid strategy
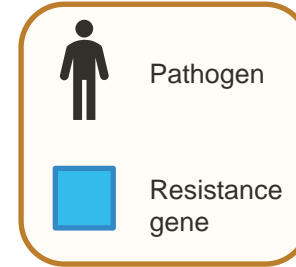
Disease resistance stewardship
- *How* we use resistance genes strongly influences *how long* we can enjoy their value
- Resistance genes are a *finite resource*
- **Stewardship**: thought and planning required to preserve effectiveness into the future
- **Pyramiding:** Use several, complementary genes
- **Farmers:** use varieties with *durable* resistance

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- Application of MAS
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    - → Major-gene selection
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  - What to select, when
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- **Opportunities for MAS in rice**
  - NTDD products
  - Blast resistance

A bar chart titled with y-axis "Proportion of QTLs" (0% to 60%) and x-axis "Frequency (elite indica)" with categories <5%, 5-25%, 25%-90%, >90%. The <5% bar reaches 50%, 5-25% reaches ~15%, 25%-90% reaches ~21%, >90% reaches ~14%. Gene names listed near the <5% bar: Pi9, Pik-h, Pish, xa13, Xa21, Xa23, Bph9-9, rymv1, rymv2, RYMV3, ...etc.
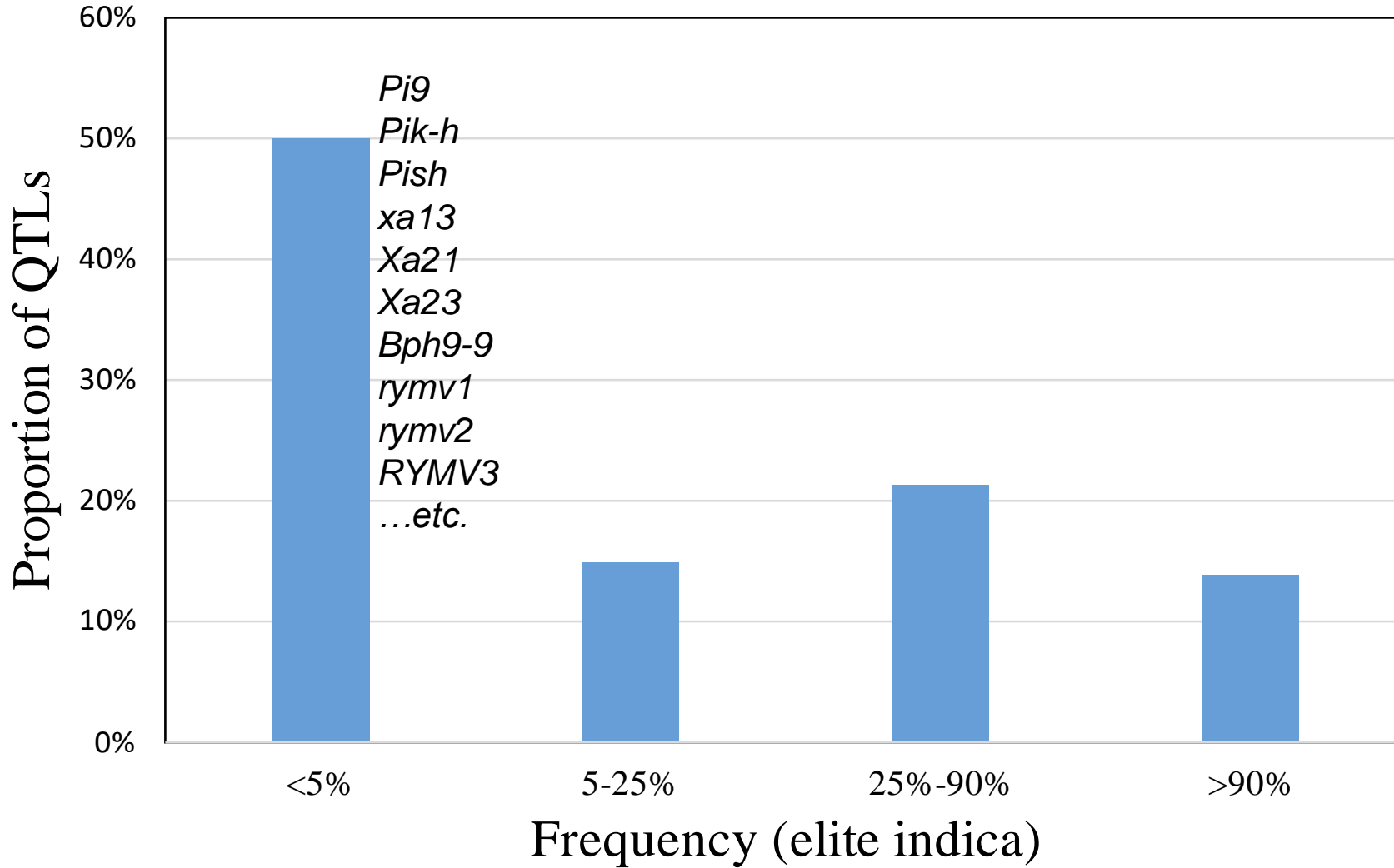
- Many genes are known, but not used successfully
- Significant opportunities for substantial progress at little cost
- Where can they be sourced from?

Leveraging the genebank: Introducing new genes
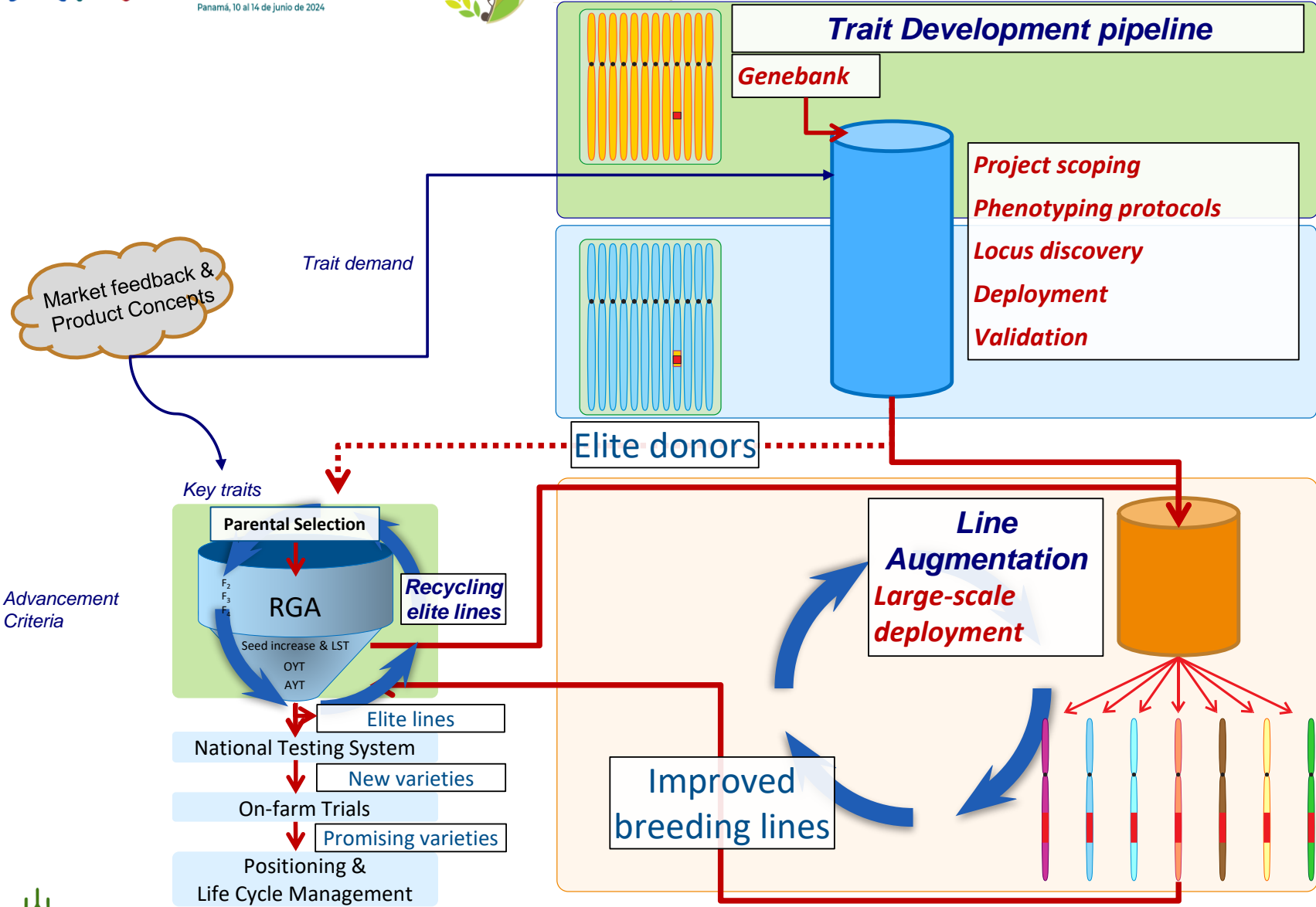
2-stage process
➢ Deployment: Quality
➢ Augmentation: Quantity

Introducing new genes

- *Yellow*: completed products (>50 genes/QTLs)
  - ➢ >95% recipient recovery
  - ➢ <2cM introgression size (red bar: to scale)
- Elite IRRI 154 background

Native trait deployment products

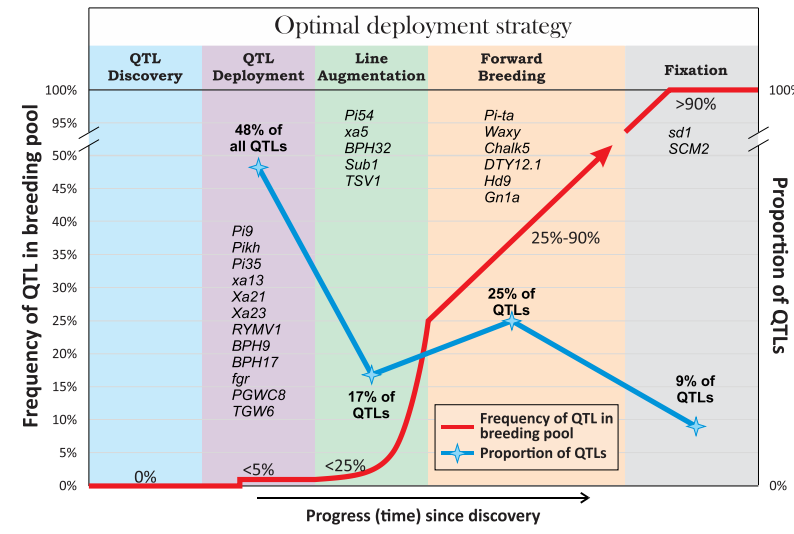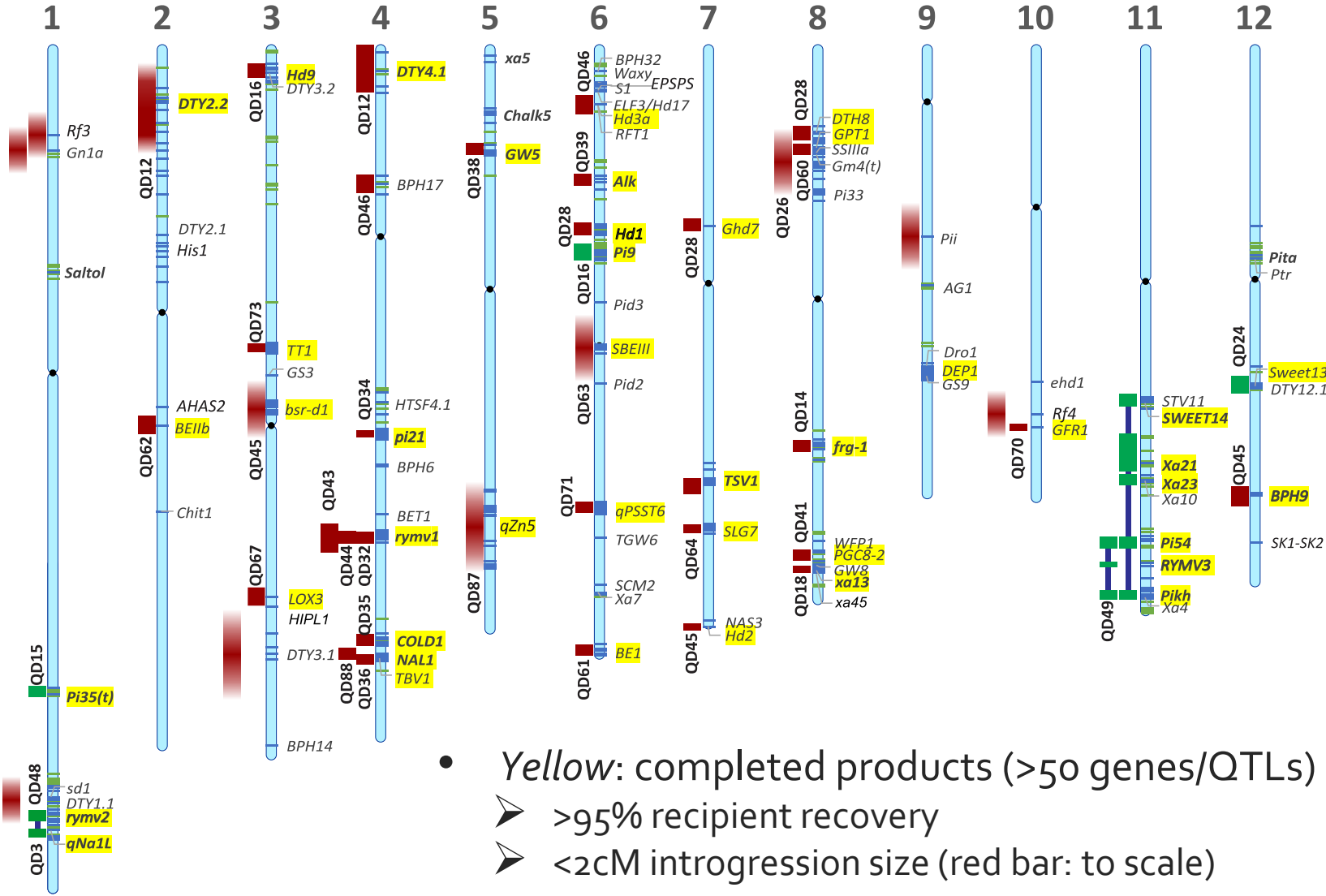| Trait	Gene	Desig-nation	Current freq.	RPP	Size	Trait	Gene	Desig-nation	Current freq.	RPP	Size
Blast (+ *Pita*)	*Pi9 (+Hd9^{N22})*	**IR20X1002**	0%	99%	<600kb	Quality (+ *Chalk5, GS3*)	*GW5*	**IR19X1006**	0%	98%	<282kb
	Pikh	**IR20X1007**	0%	97%	<462kb		*fgr-1*	**IR20X1004**	8%	99%	<209kb
	Pi54(Ttp)	**IR19X1004**	15%	100%	<475kb		*Alk^{3b}*	**IR19X1005**	12%	99%	<160kb
	Pi54(Katy)	**IR19X1003**	0%	100%	<373kb		*PGC8.2*	**IR22X1008**	0%	99%	
	Pii	**IR22X1019**	21%	97%			*Wx(int)*	**IR20X1019**	35%	97%	
	pi21	**IR22X1002**	0%	97%	<130kb		*SLG7*	**IR22X1003**	13%	100%	
BLB (+ *Xa4, xa5*)	*xa4[-] Xa5 [-]*	**IR20X1009**	0%	98%	N/A	Yield	*GNP1*	**IR22X1015**	5%	98%	<83kb
	xa13	**IR20X1006**	0%	99%	<294kb		*GFR1*	**IR22X1031**	78%	99.5%	<208kb
	Xa23	**IR21X1020**	0%	99%	<350kb		*NAL1*	**IR20X1012**	1%	99%	<279kb
	Xa21-Xa23	**IR21X1019**	0%	99%	N/A	Salinity	*qSIS1.2*	**IR22X1010**	0%	98%	<200kb
RYMV	*rymv1-2*	**IR20X1008**	0%	98%	<311kb	Drought	*qDTY2.2+ qDTY4.1*	**IR22X1006**	0%	100%	NA
	rymv1-3	**IR23X1001**	0%	100%	<595kb		*qDTY1.1 + qDTY3.1*	**TBD**	0%		
	rymv1-5	**IR23X1002**	0%	100%	<222kb	Maturity	*Hd9-N22 (DTY3.2)*	**IR20X1001**	0%	99%	<213kb
	rymv2	**IR22X1005**	0%	99%	<327kb	Heat	*TT1*	**IR23X1009**	0%	98%	<277kb
	RYMV3	**IR22X1007**	0%	99%	<238kb	Cold	*COLD1*	**IR19X1001**	0%	99%	<334kb
	rymv2 RYMV3	**IR22X1021**	0%	99%	NA	Pyramids	*sweet14-Xa21-Xa23+sweet13*	**IR22X1020**	0%	99%	N/A
BPH	*BPH32^{-}*	**IR19X1002**	58%	96%			*Pi9+Pi35+Pi54-Pikh+fgr+Hd9^{N22}*	**IR22X1018**	0%	99%	N/A
	BPH9-9	**IR22X1009**	0%	97%	<450kb						
	BPH17	TBD	10%	99%	<630kb						

➤ Fixed lines, for parents or validation

➤ Available for public breeding programs

https://docs.google.com/spreadsheets/d/14zoNdSJ61TwoeeIoXx6y-vF_V2UdbyajOQUBEWSNXF0/edit#gid=0

Deployment products: testing of blast genes

Leaf blast		IRRI BioNet												Corteva Ranchi	Advanta	Seed-Works
LINE	Allele	MO15-6	MO15-7	MO15-51	MO15-25	MO15-1	MO15-100	MO15-72	MO15-196	MO15-25	MO15-1	MO15-13	MO15-52			
IR20X1002	Pi9	R	R	R	R	R	R	R	R-M	R	R	R	M	R	M	R
IR20X1007	Pikh-Leah	M	S	R	R	R	R	R	R	R	R	R	S	R	M	M
IR20X1003	Pi35	R	R	R	M	R	R	R	M	M	R	R	R	R	M	R
IR19X1004	Pi54-Katy	M	M	S	S	M	S	R	S	S	M	M	S	M	R	R
IR19X1003	Pi54-Tetep	M	M	S	S	S	S	R	S	S	S	S	R	M	M	R
IR19X1009	Pikh+Alk	M	S	R	R	R	R	R	R	R	R	M	M	M	M	M
IRRI 154		M	S	S	S	S	S	M	S	S	S	M	M	M	M	R

Leaf blast	Allele	PO6-6	V850256	MO15-51	MO15-196	M39-1-3-8-1	BN111	MO15-6	CA89	JMB8401	BN209	CA41	JMB840610	M64-1-3-9-1	M015-23	M101-1-2-9-1	MO15-26
IR22X1018	Pi9+Pi35+Pikh-Pi54, fgr, Hd9(N22)	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
IR22X1002	pi21	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
IRRI154	Pita	S	S	S	S	M	M	M	M	R-M	R	R	R	R	R	R	R
LTH		S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	R
CO39		S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S

- No penalty observed for isolates that IRRI 154 is resistant to

- Expanded spectrum of resistance in *Pi9, Pikh* and *Pi35* NILs

- Resistance spectrum in *Pi9, Pikh* and *Pi35* NILs complementary; potential for pyramiding to improve robustness

	LINE	Locus	Allele	MO15-8	MO15-11	MO15-15	MO15-19	MO15-26	MO15-49	MO15-53	MO15-149	MO15-27	MO15-19	MO15-26	MO15-53	MO15-14	MO15-41	MO15-47	MO15-68
B1	IR20X1002	Pi9/Pi2/Piz-t locus	Pi9	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
B2	IR20X1007	Pik locus	Pikh-Leah	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
B3	IR20X1003	Os01g0782100	Pi35	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
B4	IR19X1004	Pi54	Pi54-Katy	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
B5	IR19X1003	Pi54	Pi54-Tetep	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
B6	IR20X1005	SSIIa (Alk)	Alk-3b	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
B7	IR20X1006	xa13	xa13	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
B8	IR20X1008	qSIS1.2		R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
B9	IR19X1002	BPH32	[-]	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
B10	IR19X1006	GW5	GW5-Lemont	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
B11	IR20X1004	BADH2	fgr-1	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
B12	IR19X1005	SSIIa (Alk)	Alk-3b	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	M
B13	IR20X1001	DTY3.2	qDTY3.2-N22	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
B14	IR19X1001	COLD1	COLD1	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
B15	IR19X1008	rymv1	rymv1-2	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
B16	IR19X1007	salinity pyramid		R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
B17	IR19X1009	-Pikh + Alk	Pikh-Leah	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
B18	IRRI 154			R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
	LTH			S	S	S	S	S	R	R	S	S	S	S	S	S	S	S	S
	CO 39			R	S	S	R	S	R	R	R	S	S	R	S	S	R	R	S

Deployment products: testing of blast genes

Leaf blast	Allele	PO6-6	V850256	MO15-51	MO15-196	M39-1-3-8-1	BN111	MO15-6	CA89	JMB8401	BN209	CA41	JMB840610	M64-1-3-9-1	M015-23	M101-1-2-9-1	MO15-26
IR22X1018	Pi9+Pi35+Pikh-Pi54, fgr, Hd9(N22)	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
IR22X1002	pi21	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
IRRI154	Pita	S	S	S	S	M	M	M	M	R-M	R	R	R	R	R	R	R
LTH		S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	R
CO39		S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S

Neck blast	Allele	M101-1-2-9-1	BN209	PO6-6	MO15-19	V86010	CA41	M36-1-3-10-1	BN111	M64-1-3-9-1	V850256
IR22X1018	Pi9+Pi35+Pikh-Pi54, fgr, Hd9(N22)	R	R	R	R	R	R	R	R	R	R
IR22X1002	pi21	M	M	S	S	R/S	M	M	S	M	M
IRRI154	Pita	M	M	S	S	R	S	S	S	S	S
CO 39 (S CHECK)		S	S	S	S	S	S	S	S	S	S

- Pyramiding does improve robustness/durability
- Effective against all tested isolates, in all tested locations (so far)
- Durable strategy for blast resistance across many countries/regions
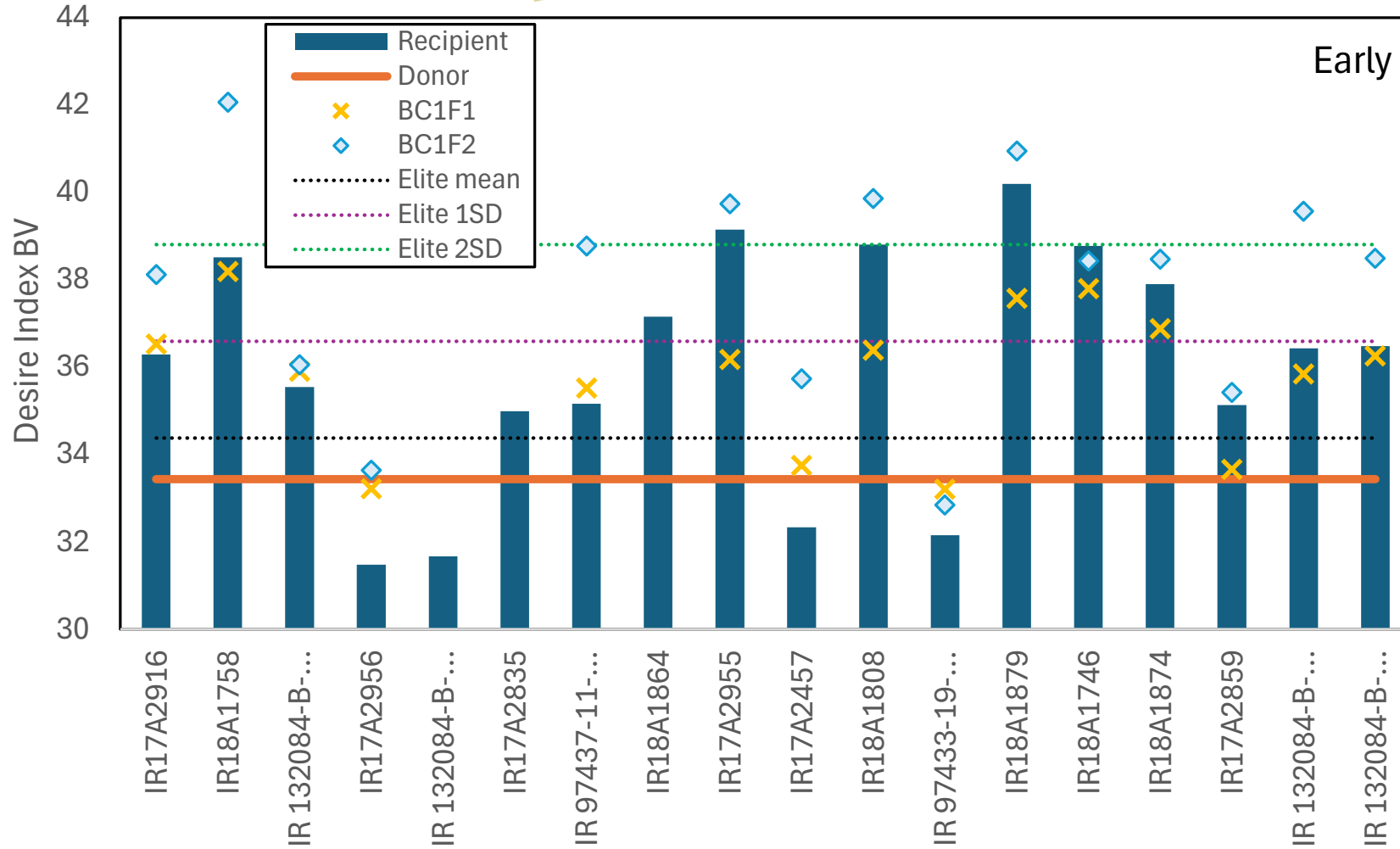
Blast: Upscaling availability

➤ Pyramid is focus of 2023/2024 augmentation activities

➤ 65 recipients from IRRI and AfricaRice breeding programs

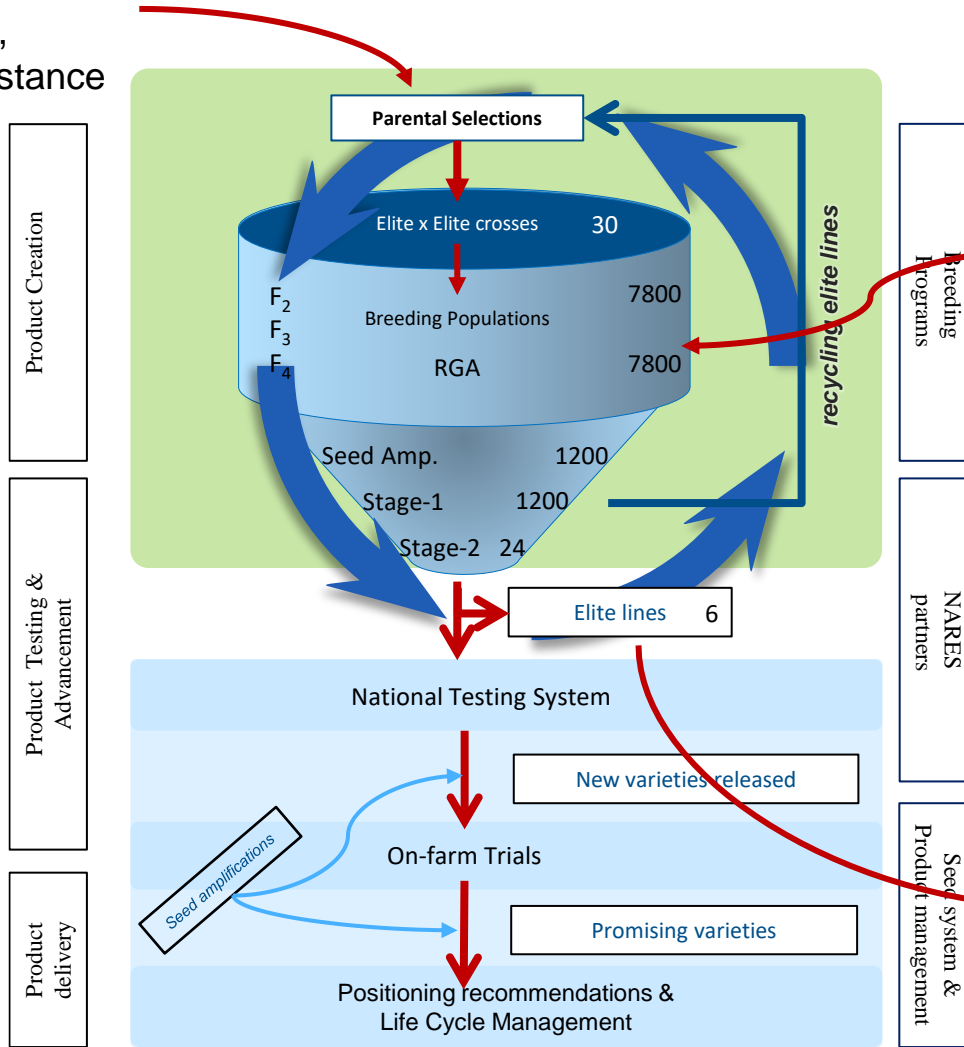Blast: Upscaling availability - BC1F$_2$ selections

- GEBV at BC1F$_1$ showed no relationship with RPP
  - No major penalties introduced from the donor
- Selection based on GEBV is reliably identifying segregants as good or better than recipient varieties
- In most cases these are over 1SD above the mean, in some cases over 2SD
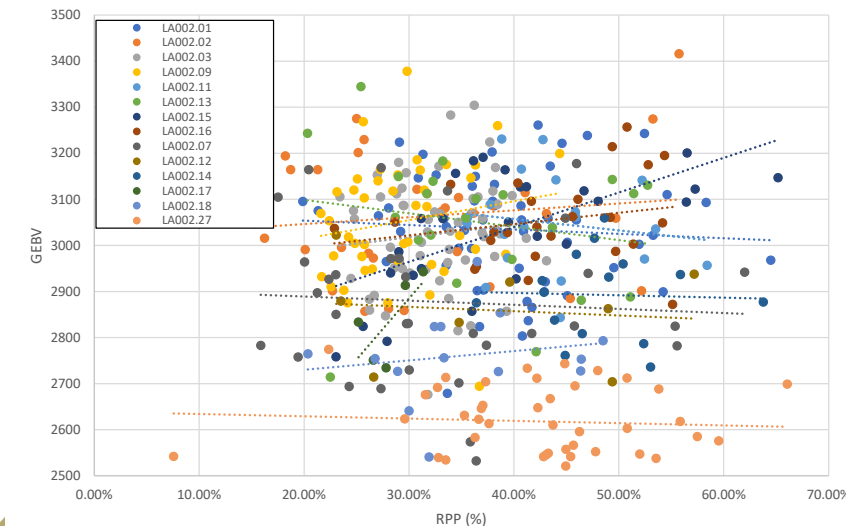
Blast: Application in breeding

Deployment Augmentation
High-quality, durable resistance

Intertek markers
Cost-efficient
Reliable across rice diversity

Better varieties
Stronger, more durable resistance
Better performance vs. using landrace donors

F=5%, R=20	1-stage selection	2-stage selection	
Q (# Loci)	Fixation at F4	F2 enrichment, fixing 1 gene	F4 fixation, 1 gene fixed
1	60	107	
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Thanks!