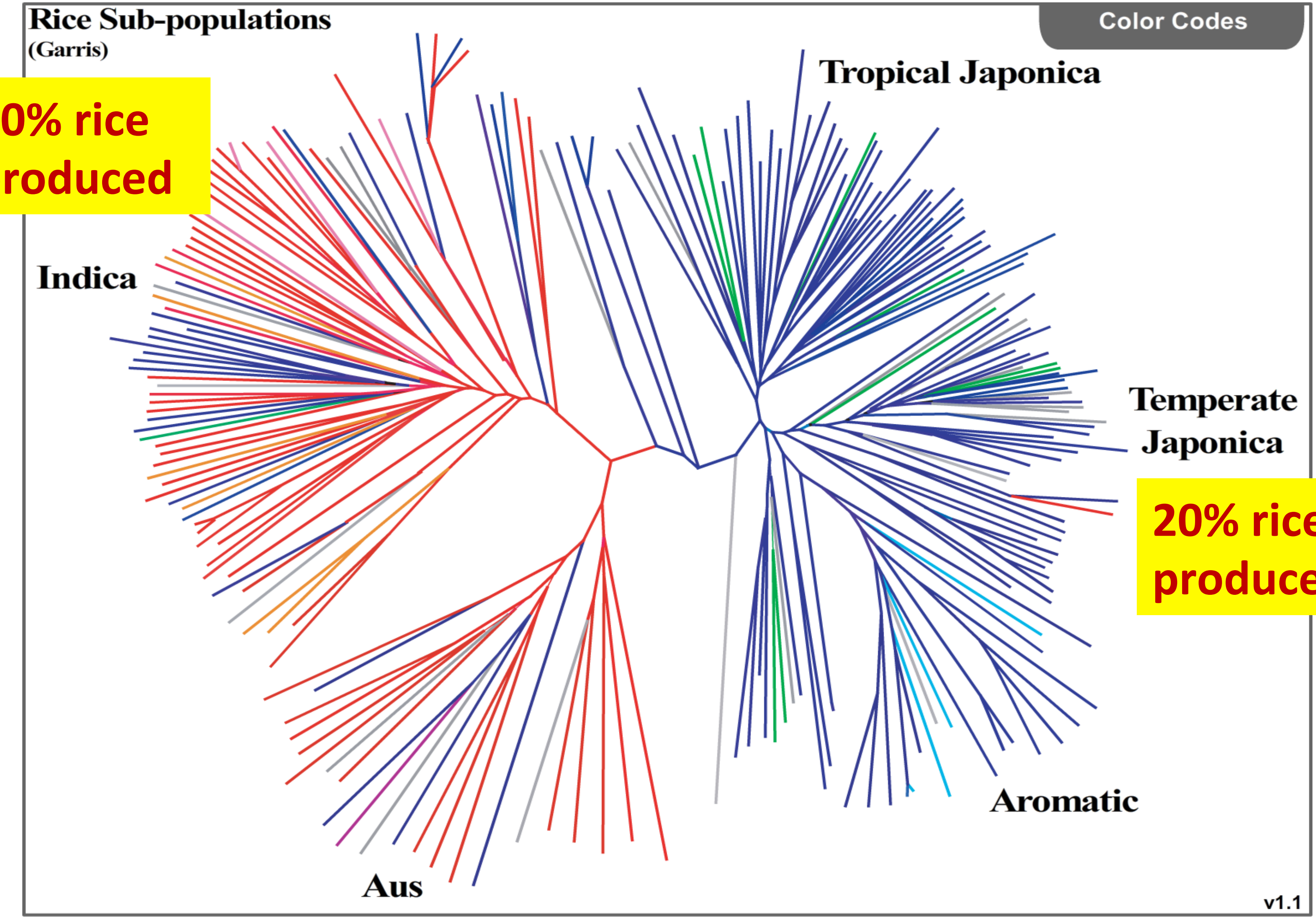
Major Types of Rice



80% rice produced

20% rice produced

http://www.ricediversity.org/garrisfigure/

Design: Selection of founding individuals

- Breeding relevance – elite varieties – high yielding, preferred grain quality,
- a combination of resistance/tolerance to biotic and abiotic stress
- Capture diversity over different rice-growing environments

MAGIC *indica*


High yielding and most popular variety of the Philippines

IRRI

Oryza sativa var. PSBRC 82
Origin:


Blast resistant, high yielding

China

Oryza sativa var. SHZ
Origin:


stay green/delayed senescence

Colombia

Oryza sativa var. Fedearroz 50
Origin:


Drought tolerant in lowlands tungro resistance

IRRI

Oryza sativa var. IR77298
Origin:


High yielding variety in New Plant Type II background

Oryza sativa var. NSIC RC 158
Origin: IRRI

Indica / tropical japonica background


salt tolerant at seedling and reproductive stages

IRRI

Oryza sativa var. IR 46-30
Origin:


Fe toxicity tolerant

Oryza sativa var. IR45427
Origin:

IRRI

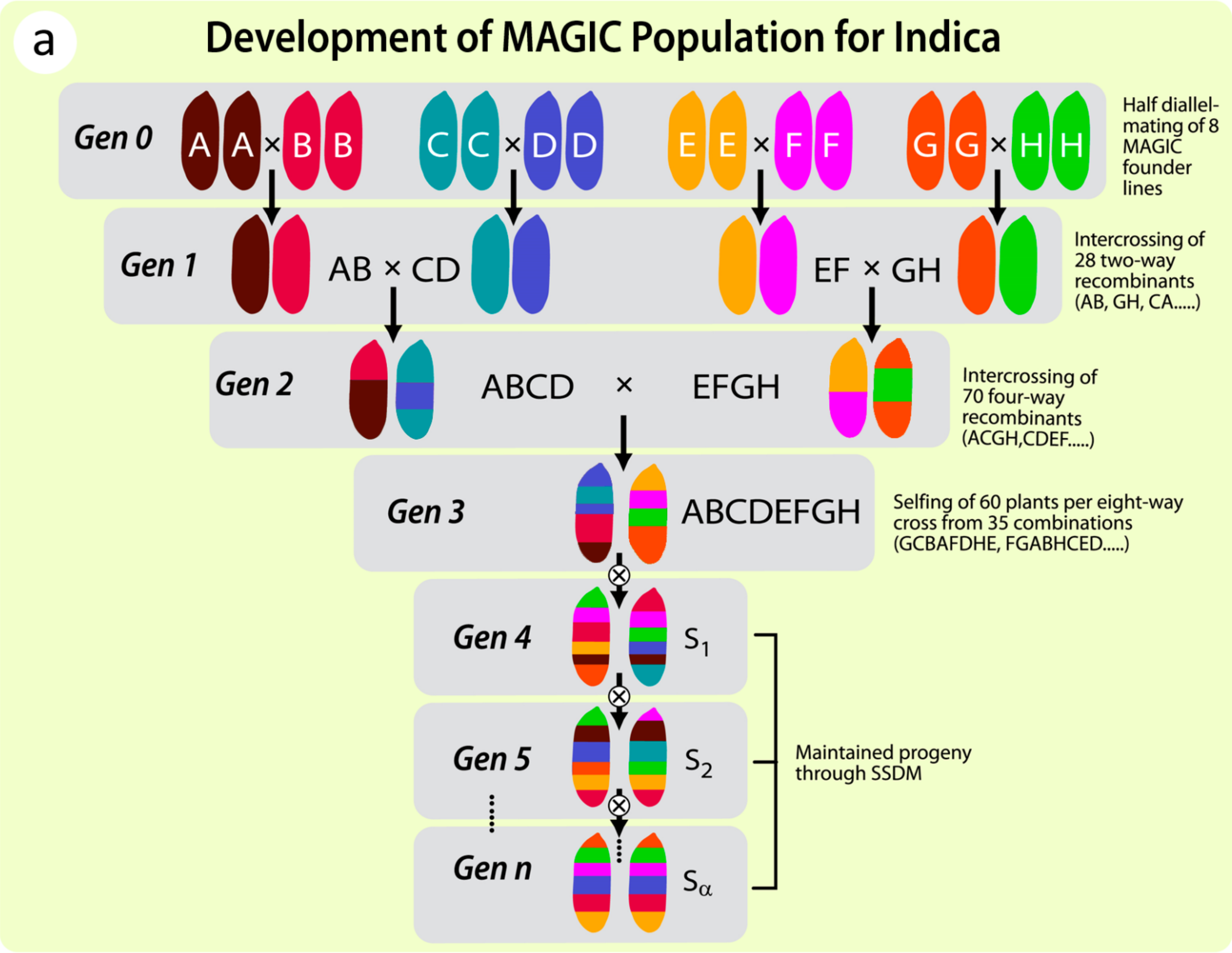

Mega variety, excellent grain quality and submergence tolerance

India (IRRI)

Oryza sativa var. Samba Mashuri
Origin:

- PSBRc82
- Sanhuangzhan-2
- Fedearroz 50
- IR77298-14-1-2-10
- PSBRc 158
- IR4630-22-2-5-1-3
- IR45427-2B-2-2B-1-1
- Sambha Mahsuri + Sub1

Design: Structure and size of the population *indica* MAGIC and *japonica* MAGIC



Development of MAGIC Population for Indica

Half diallel-mating of 8 MAGIC founder lines

Intercrossing of 28 two-way recombinants (AB, GH, CA.....)

Intercrossing of 70 four-way recombinants (ACGH,CDEF.....)

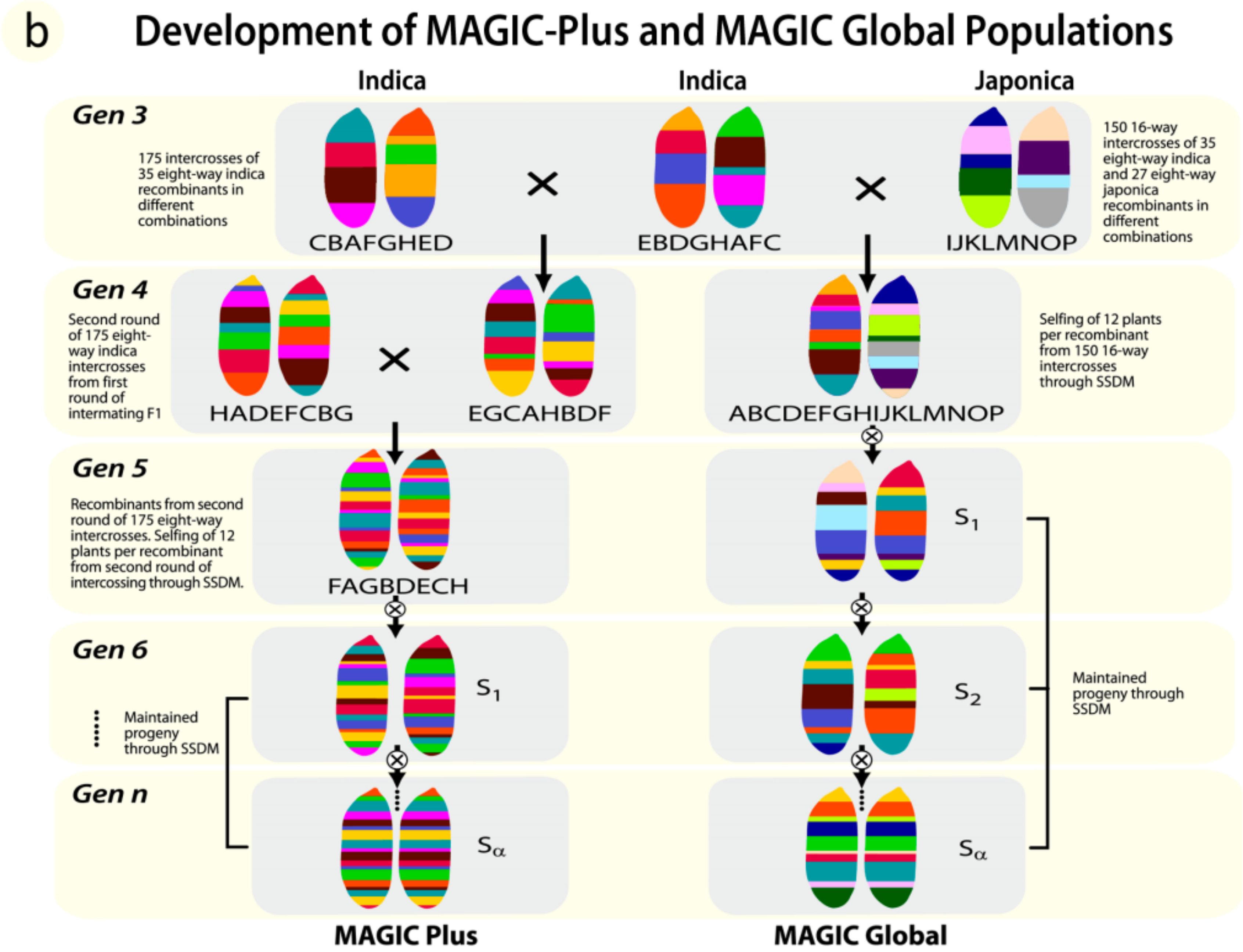
Selfing of 60 plants per eight-way cross from 35 combinations (GCBAFDHE, FGABHCED.....)

Maintained progeny through SSDM

Indica MAGIC development 2008-2009

- 1st crossing cycle - **28 single intercrosses** in a half-diallel approach
  - E.g.: A/B, C/D, E/F, A/H,…. **28**
- 2nd crossing cycle - each single intercross was inter-mated to generate **70 four-way** crosses out of all possible 210 crosses
  - E.g.: A/B//D/C, ~~CD//BA~~, F/A//G/C,….70
- 3rd crossing cycle - **35 out of 105 possible eight-way** crosses with each founder line being represented once
  - E.g.: A/H//C/G///E/F//B/D

A balance was sought by trying to use every parent 3-4 times as male and 3-4 times as female



Development of MAGIC-Plus and MAGIC Global Populations

175 intercrosses of 35 eight-way indica recombinants in different combinations

150 16-way intercrosses of 35 eight-way indica and 27 eight-way japonica recombinants in different combinations

Second round of 175 eight-way indica intercrosses from first round of intermating F1

Selfing of 12 plants per recombinant from 150 16-way intercrosses through SSDM

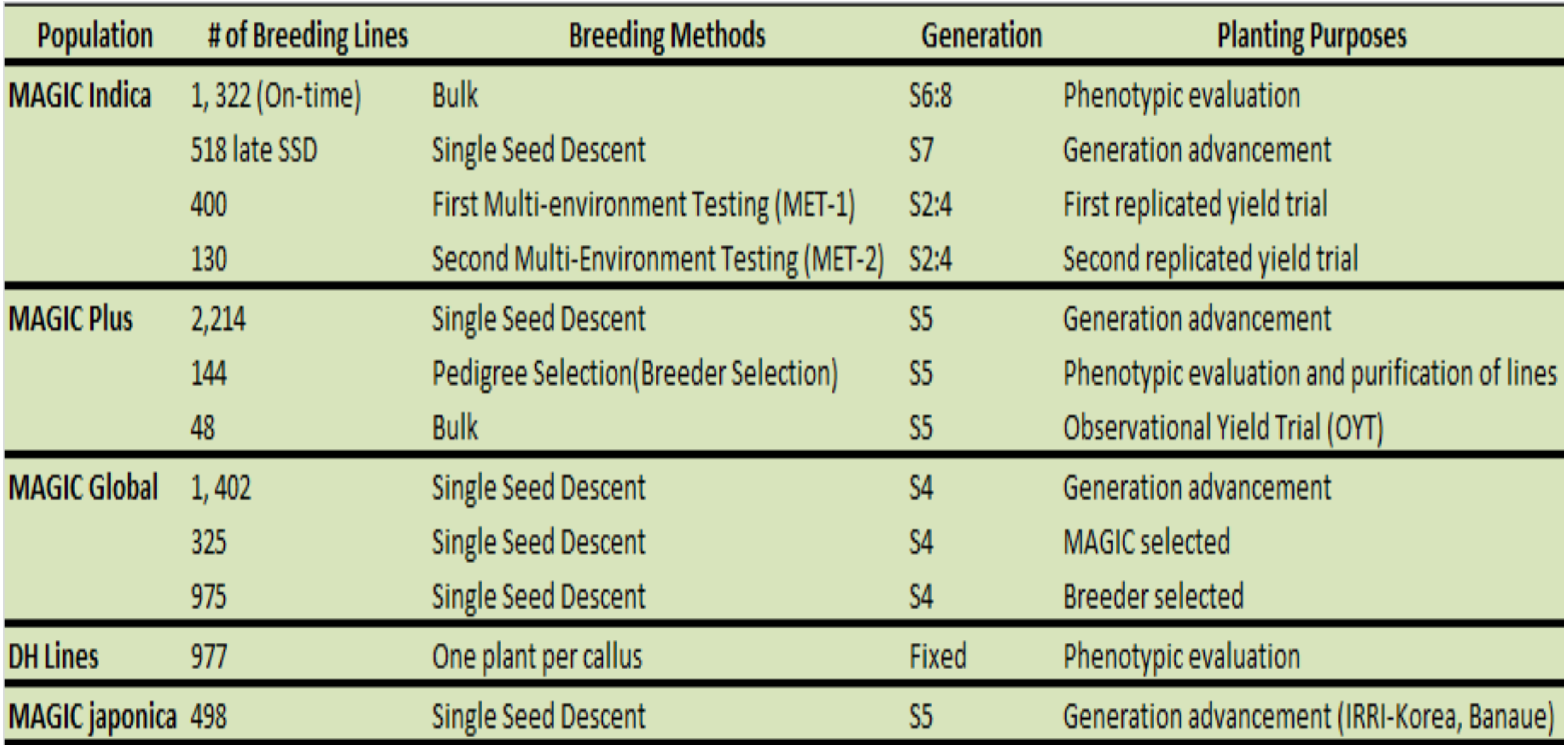
Recombinants from second round of 175 eight-way intercrosses. Selfing of 12 plants per recombinant from second round of intercossing through SSDM.

Maintained progeny through SSDM

Maintained progeny through SSDM

Design: Size and status of the MAGIC population in Rice

CURRENT STAUTS OF MAGIC in Rice♪

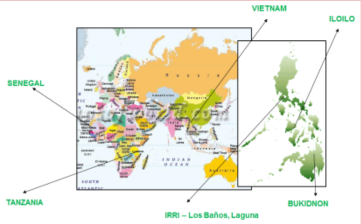


Population	# of Breeding Lines	Breeding Methods	Generation	Planting Purposes
MAGIC Indica	1, 322 (On-time)	Bulk	S6:8	Phenotypic evaluation
	518 late SSD	Single Seed Descent	S7	Generation advancement
	400	First Multi-environment Testing (MET-1)	S2:4	First replicated yield trial
	130	Second Multi-Environment Testing (MET-2)	S2:4	Second replicated yield trial
MAGIC Plus	2,214	Single Seed Descent	S5	Generation advancement
	144	Pedigree Selection(Breeder Selection)	S5	Phenotypic evaluation and purification of lines
	48	Bulk	S5	Observational Yield Trial (OYT)
MAGIC Global	1, 402	Single Seed Descent	S4	Generation advancement
	325	Single Seed Descent	S4	MAGIC selected
	975	Single Seed Descent	S4	Breeder selected
DH Lines	977	One plant per callus	Fixed	Phenotypic evaluation
MAGIC japonica	498	Single Seed Descent	S5	Generation advancement (IRRI-Korea, Banaue)

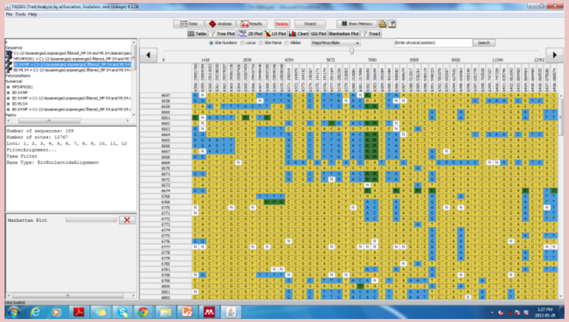
Design: How the population complements existing resources (in particular, other multi-parental populations)

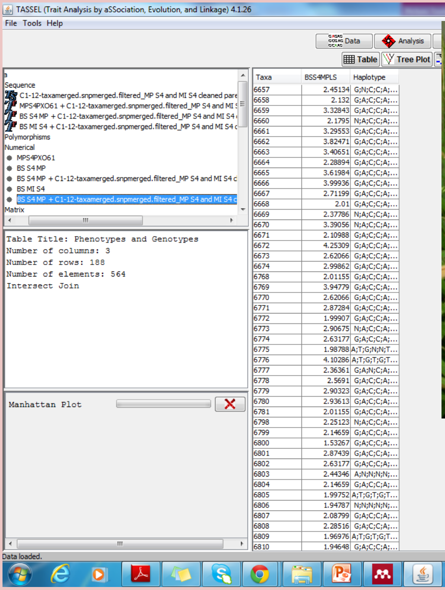
- **Diversity Panel from International Rice GeneBank–**
  ~5,000 re-sequenced or SNP genotyped germplasm
- **Bi-parental RILs** - used for gene mapping
- **Near-Isogenic Lines (NILs) and gene pyramids**
- **Elite and released varieties** from pedigree breeding programs

MAGIC
Multiple QTLs in individual MAGIC lines



DATA





Sequence
C1-12-taxamerged.snpmerged.filtered_MP S4 and MI S4 cleaned pare
MPS4PXO61 + C1-12-taxamerged.snpmerged.filtered_MP S4 and MI S
BS S4 MP + C1-12-taxamerged.snpmerged.filtered_MP S4 and MI S4
BS MI S4 + C1-12-taxamerged.snpmerged.filtered_MP S4 and MI S4 c
Polymorphisms
Numerical
- MPS4PXO61
- BS S4 MP
- BS S4 MP + C1-12-taxamerged.snpmerged.filtered_MP S4 and MI S4 c
- BS MI S4
- BS S4 MP + C1-12-taxamerged.snpmerged.filtered_MP S4 and MI S4 c

Matrix

```
Table Title: Phenotypes and Genotypes
Number of columns: 3
Number of rows: 188
Number of elements: 564
Intersect Join
```

Taxa	BSS4MPLS	Haplotype
6657	2.45134	G;N;C;C;A;...
6658	2.132	G;A;C;C;A;...
6659	3.32843	G;A;C;C;A;...
6660	2.1795	N;A;C;C;A;...
6661	3.29553	G;A;C;C;A;...
6662	3.82471	G;A;C;C;A;...
6663	3.40651	G;A;C;C;A;...
6664	2.28894	G;A;C;C;A;...
6665	3.61984	G;A;C;C;A;...
6666	3.99936	G;A;C;C;A;...
6667	2.71199	G;A;C;C;A;...
6668	2.01	G;A;C;C;A;...
6669	2.37786	N;A;C;C;A;...
6670	3.39056	N;A;C;C;A;...
6671	2.10988	G;A;C;C;A;...
6672	4.25309	G;A;C;C;A;...
6673	2.62066	G;A;C;C;A;...
6674	2.99862	G;A;C;C;A;...
6768	2.01155	G;A;C;C;A;...
6769	3.94779	G;A;C;C;A;...
6770	2.62066	G;A;C;C;A;...
6771	2.87284	G;A;C;C;A;...
6772	1.99907	G;A;C;C;A;...
6773	2.90675	N;A;C;C;A;...
6774	2.63177	G;A;C;C;A;...
6775	1.98788	A;T;G;N;N;T...
6776	4.10286	A;T;G;T;G;T...
6777	2.36361	G;A;N;C;A;...
6778	2.5691	G;A;C;C;A;...
6779	2.90323	G;A;C;C;A;...
6780	2.93613	G;A;C;C;A;...
6781	2.01155	G;A;C;C;A;...
6798	2.25123	N;A;C;C;A;...
6799	2.14659	G;A;C;C;A;...
6800	1.53267	G;A;C;C;A;...
6801	2.87439	G;A;C;C;A;...
6802	2.63177	G;A;C;C;A;...
6803	2.44346	A;N;N;N;N;...
6804	2.14659	G;A;C;C;A;...
6805	1.99752	A;T;G;T;G;T...
6806	1.94787	N;N;N;N;N;...
6807	2.08799	G;A;C;C;A;...
6808	2.28516	G;A;C;C;A;...
6809	1.96976	A;T;G;T;G;T...
6810	1.94648	G;A;C;C;A;...

Data loaded.

Data: what type of information is already available - e.g., physical maps, sequence, annotation, none of the above

- **Reference genomes**
    - Nippinobare (*japonica*)-well annotated
    - IR64 (*indica*) in pipeline
- **~5,000 diverse germplasm**
    - – re-sequenced/SNP-genotyped (on-going)

Data: what kind of genotyping data you're collecting and on how much of the population

- Deep sequencing of the founder parents
- Imputing, haplotypes and increased SNP marker density to determine recombination events
- Genotyping by sequencing (GBS)
  - Replicated GBS of parents vs deep sequencing
  - Most of MAGIC lines in advanced generation (S6-S8)

Genotype data

- ***Indica* MAGIC**
  - **200 lines at S4** and 8 parents- **GBS** - data:
    - GWAS - TASSEL; interval mapping using R-mpMAP
  - **1321 lines S6:8** and 8 parents-**GBS** by August 2013

- **MAGIC PLUS**
  - **190 lines at S4** - **GBS** - data in hand - Analyzed GWAS - TASSEL; interval mapping - mpMAP

- MAGIC Multiple-Environment Trials (MET1)
  - **130 bulks (S2:4)** representing the MET 2 bulks - **GBS** data in hand - Analyzing GWAS - TASSEL

Data: phenotypes of interest, and on how much of the population they're being measured

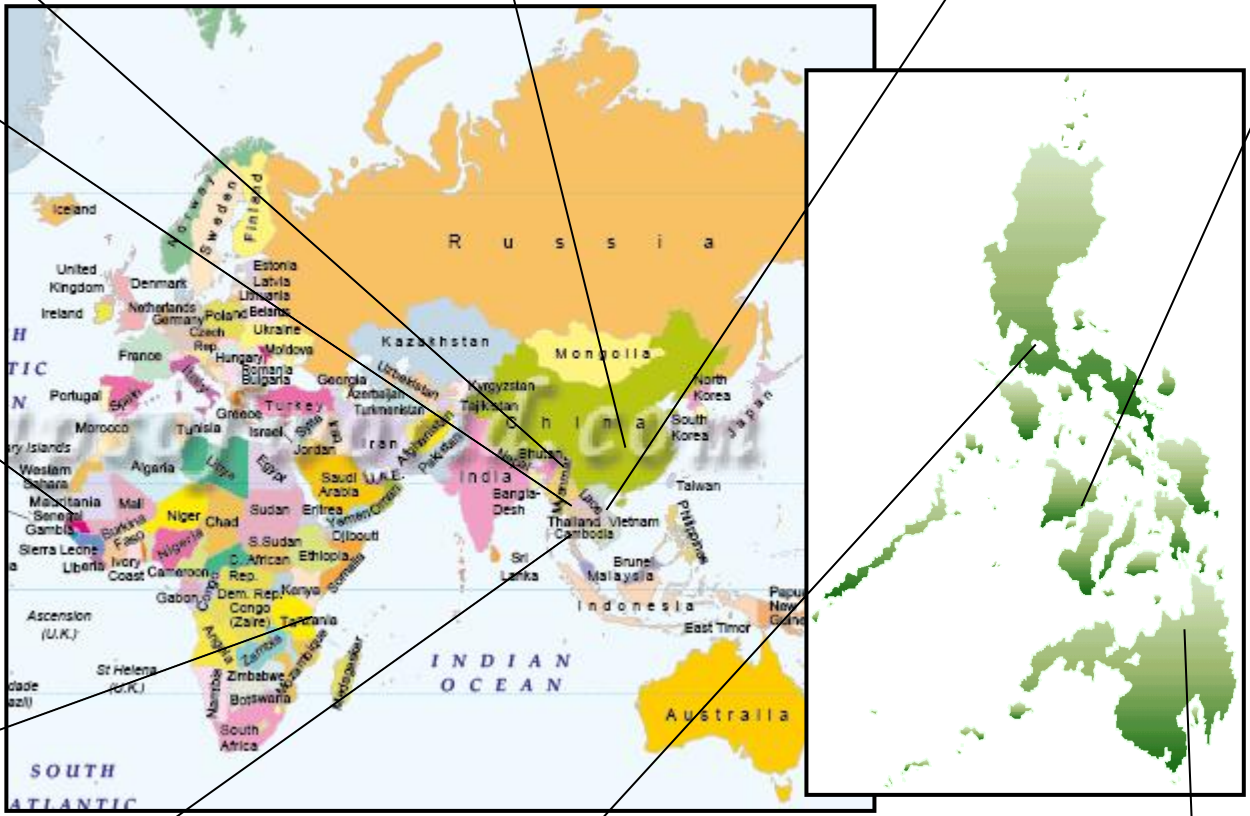
Multi-Environment Trials (MET)

- Target environments – Southeast Asia and Africa
- The network
  - Philippines (3 sites)
  - Vietnam (1 site)
  - Africa (Tanzania and Senegal)

MAGIC MET-2 Nursery 2012 WS

Collect basic agronomic traits: flowering time, tillering, yield components etc

MAGIC Multi-Environment Trials (METs)



MYANMMAR

CHINA

VIETNAM

ILOILO

THAILAND

SENEGAL

TANZANIA

CAMBODIA

IRRI – Los Baños, Laguna

BUKIDNON

On-going Trials

Additional Target Sites

Data: phenotypes of interest, and on how much of the population they're being measured

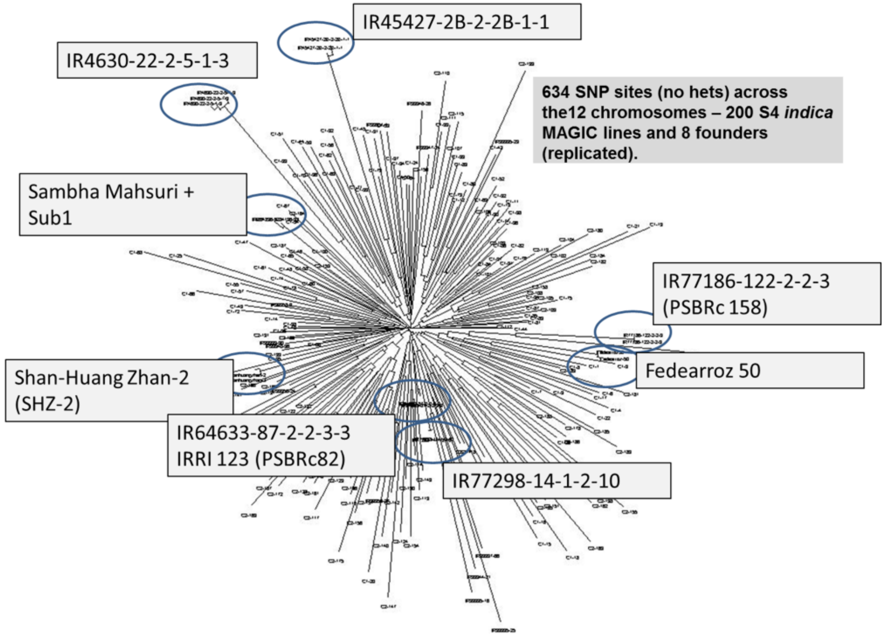
- Drought – MET1 (400) – yield under drought
- Grain quality – MET1 (400)
- *Indica* MAGIC (200) and MAGIC PLUS (190) S4
  - **Grain quality** – Grain length, width, amylose content, gelatinization temperature, chalkiness
  - **Biotic stress** – blast disease, bacterial blight, brown spot
  - **Abiotic stress** – submergence, salt tolerance

Methods -approaches

- **GWAS – TASSEL –**
  - MLM– S4 indica MAGIC (200) and MAGIC PLUS (190)
  - show associations with large-effect loci
  - smaller effect and novel loci--need to be validated

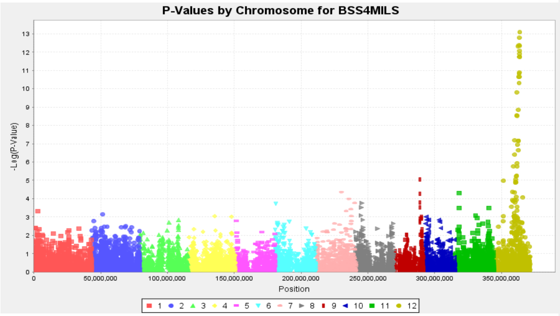
- **R-mpMAP**
  - Interval mapping -fine mapping in MAGIC PLUS
  - Comparisons MAGIC PLUS vs *indica* MAGIC:
  - Recombination events/frequency
  - SNP density. Need filtering and imputation

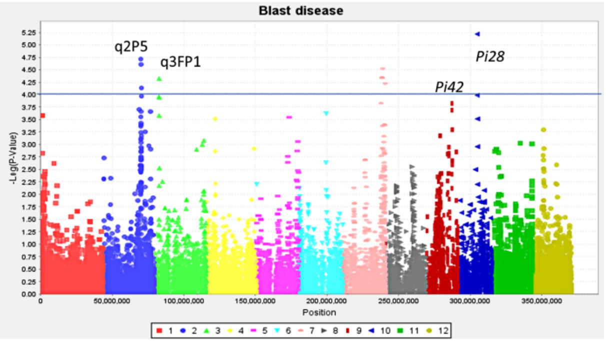
Biotic stress

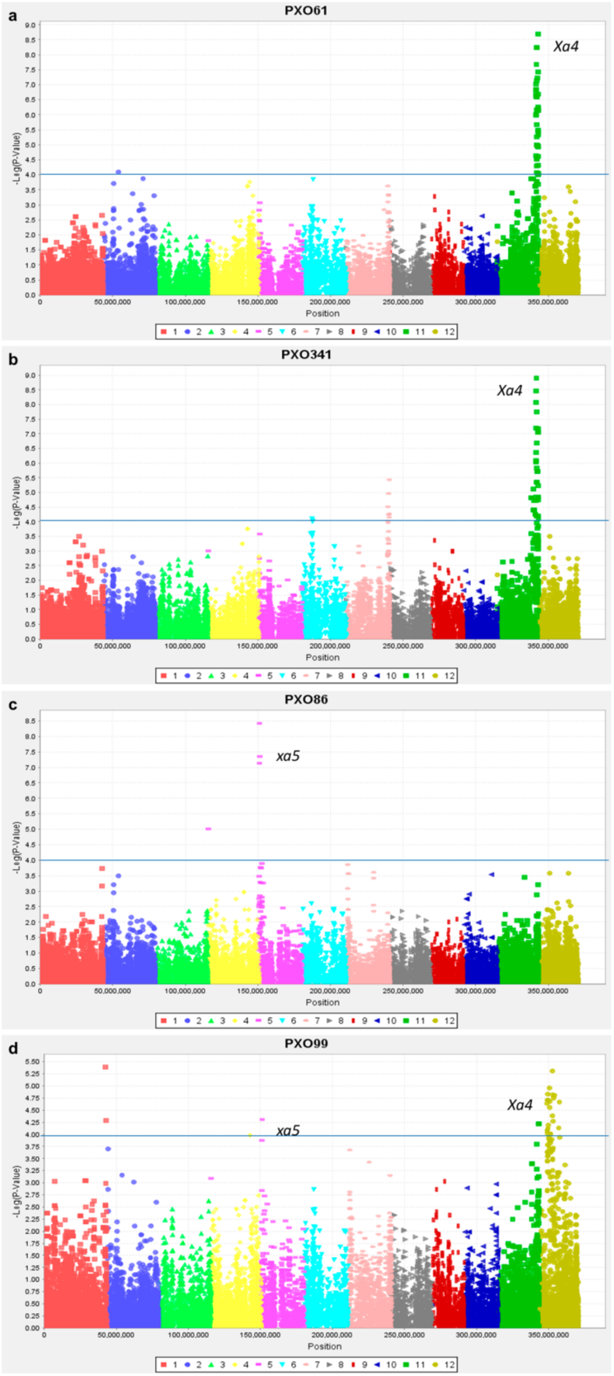


634 SNP sites (no hets) across the12 chromosomes – 200 S4 *indica* MAGIC lines and 8 founders (replicated).

CLADOGRAM

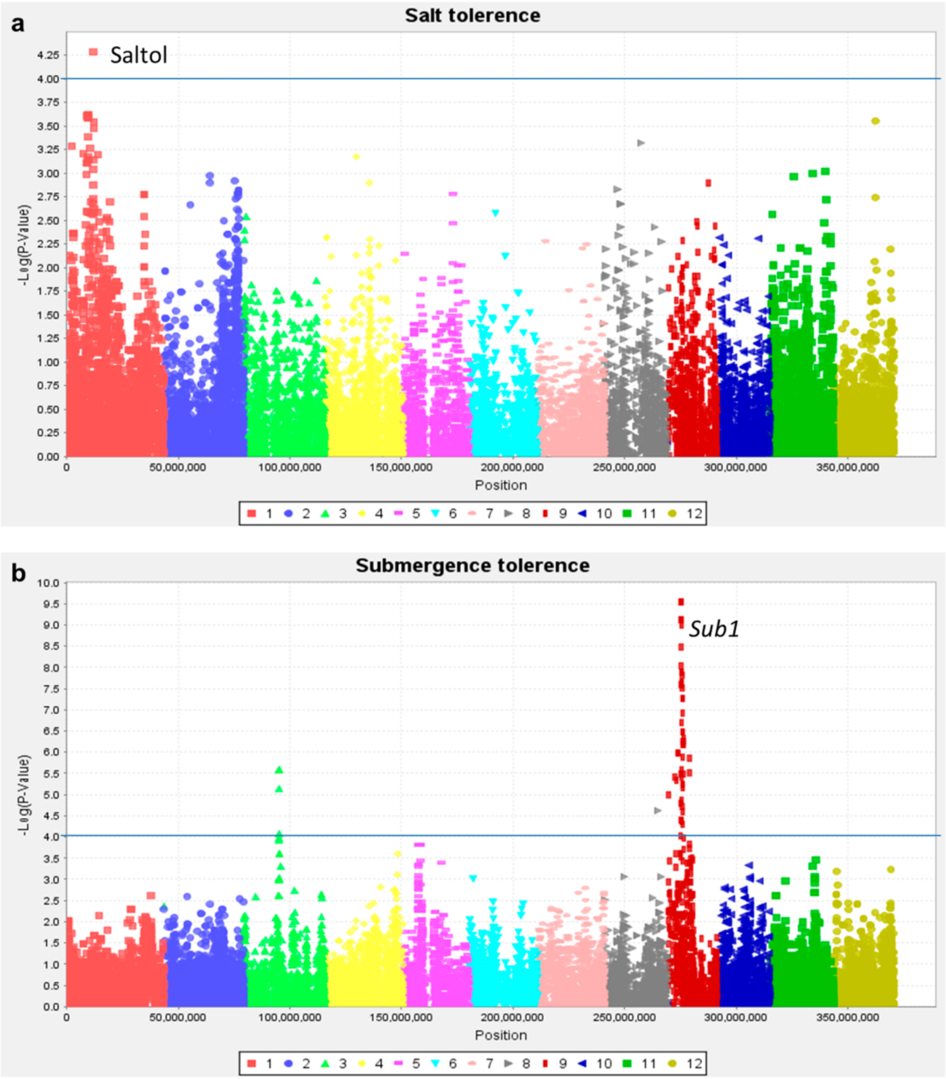


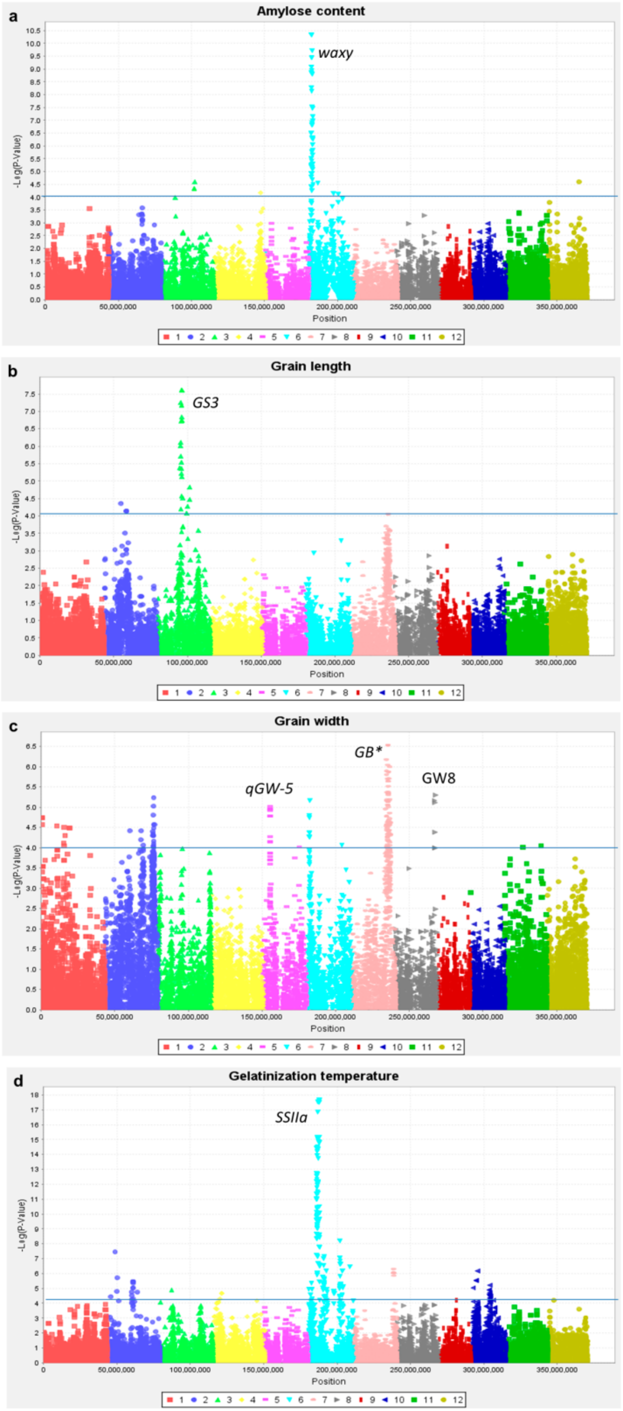




Abiotic Stress

Grain Quality

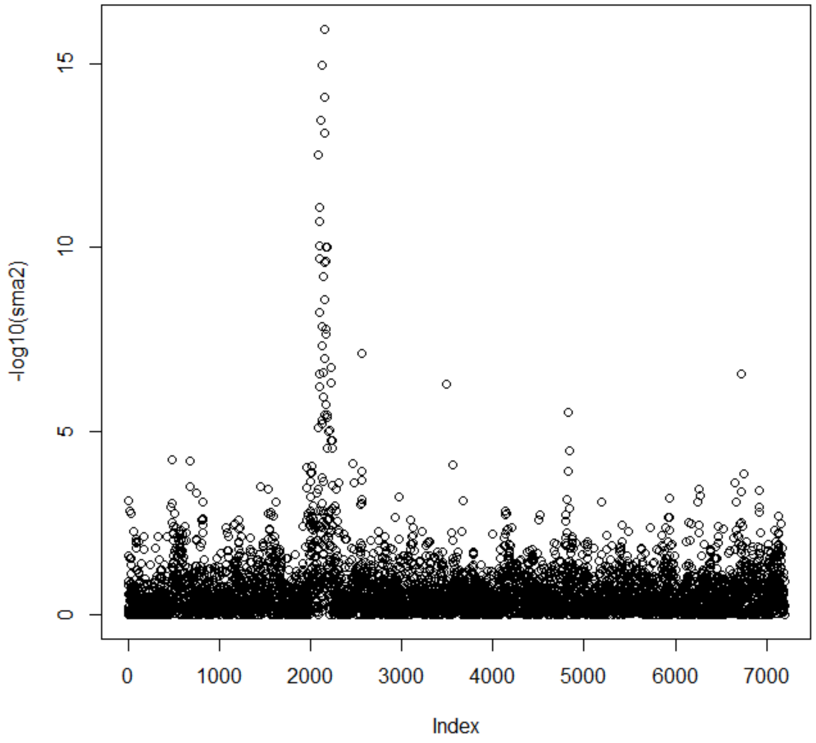




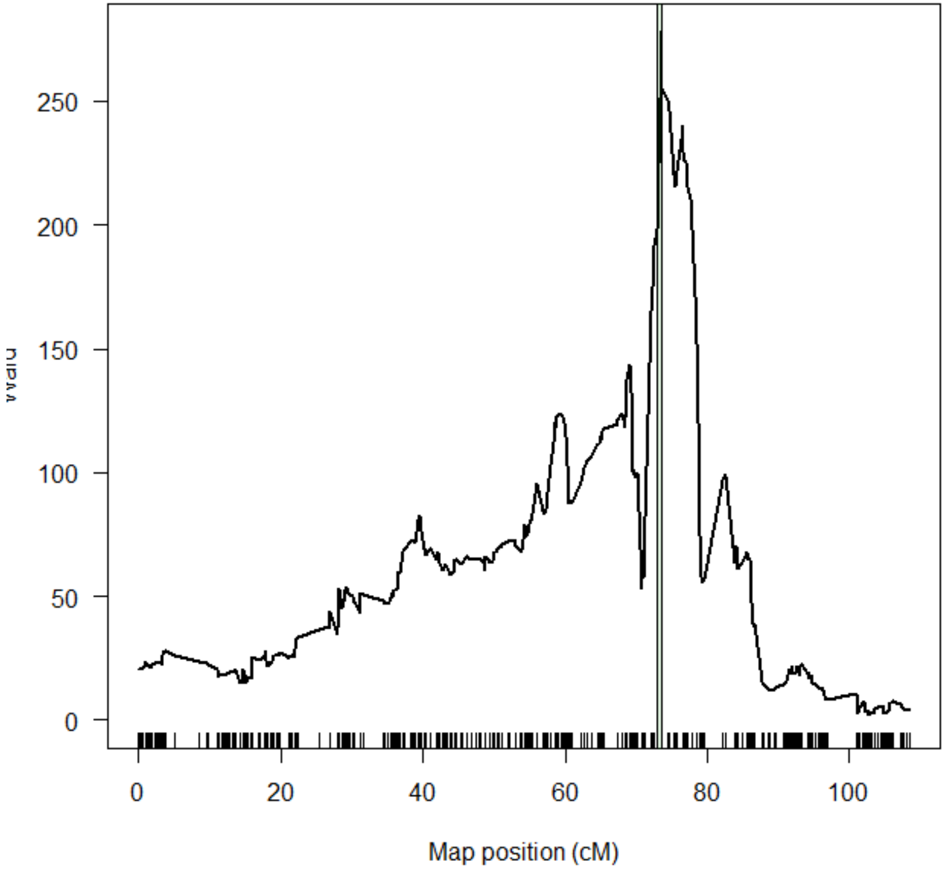
R-mpMAP

Brown spot disease

Single marker analysis



interval mapping on Chr 12



Work with Dr. Emma Huang, CSIRO

Challenges: what problems have you run into along the way?

- Imputation - GBS sparse data
- Regions of recombination
- Phenotype large populations - subset
- Physical position to cM – using factors?

Challenges : what questions would you like to answer but may not be quite sure how yet?

- Relationship between intercrosses (number of meioses) and recombination
- Resolution of mapping
- Interaction of large and small effect QTLs
- Complex traits due to small effect loci
- Degree of transgressive segregation
- Use for phenotype predictions (training set)

Challenges: looking back, is there anything you would do differently?

- Crossing scheme to ensure equal contributions of founders (seems skewed in indica MAGIC population

- For breeding, simplify crossing scheme.
  - e.g., not to make the 70 4-way followed by 35 8-ways. Just one would suffice making sure all parents are included and a large population size.

MAGIC Team

Hei Leung
Ed Redoña
Glenn Gregorio
RK Singh

Nonoy Bandillo, Chitra Raghavan

Irish Lobina
Pauline Andrea Muyco
Anna Sevilla
Cesar Caspillo
Vernie Carandang

Mike Thomson
Jade Dilla
Mayee Reveche

Ramil Mauleon
Guyou Ye
CRIL members

Hybridization Group
Phenotyping Teams at IRRI

INGER Group
Allan Salabsabin
MET Team

Cornell University
Susan McCouch, Chih-Wei Tung (now at NTU Taiwan)
Members of Ed Buckler's group
CSIRO
Emma Huang

Scholars
1 PhD Scholar (Bangladesh)
3 MS Scholar (Philippines, Myanmar)
2 BS Affiliate Scholar (Philippines)
…………….. And many more

Thanks

Fedearroz 50
/
Sanhuangzhan No 2

//

IR 77298-14-1-2-10
/
IR07F287

///

IRRI 123
/
IR 4630-22-2-5-1-3

//

IR 45427-2B-2-2B-1-1
/
NSIC RC 158

- And 35 out of 105 possible eight-way crosses in the 2009 dry season, with each founder line being represented once

35 such unique combinations

8-way

Fedearroz 50/Sanhuangzhan No 2//IR 77298-14-1- 2-10/IR07F287///IRRI 123/ IR4630-22-2-5-1-3//IR 45427-2B-2-2B-1-1/ NSIC RC 158

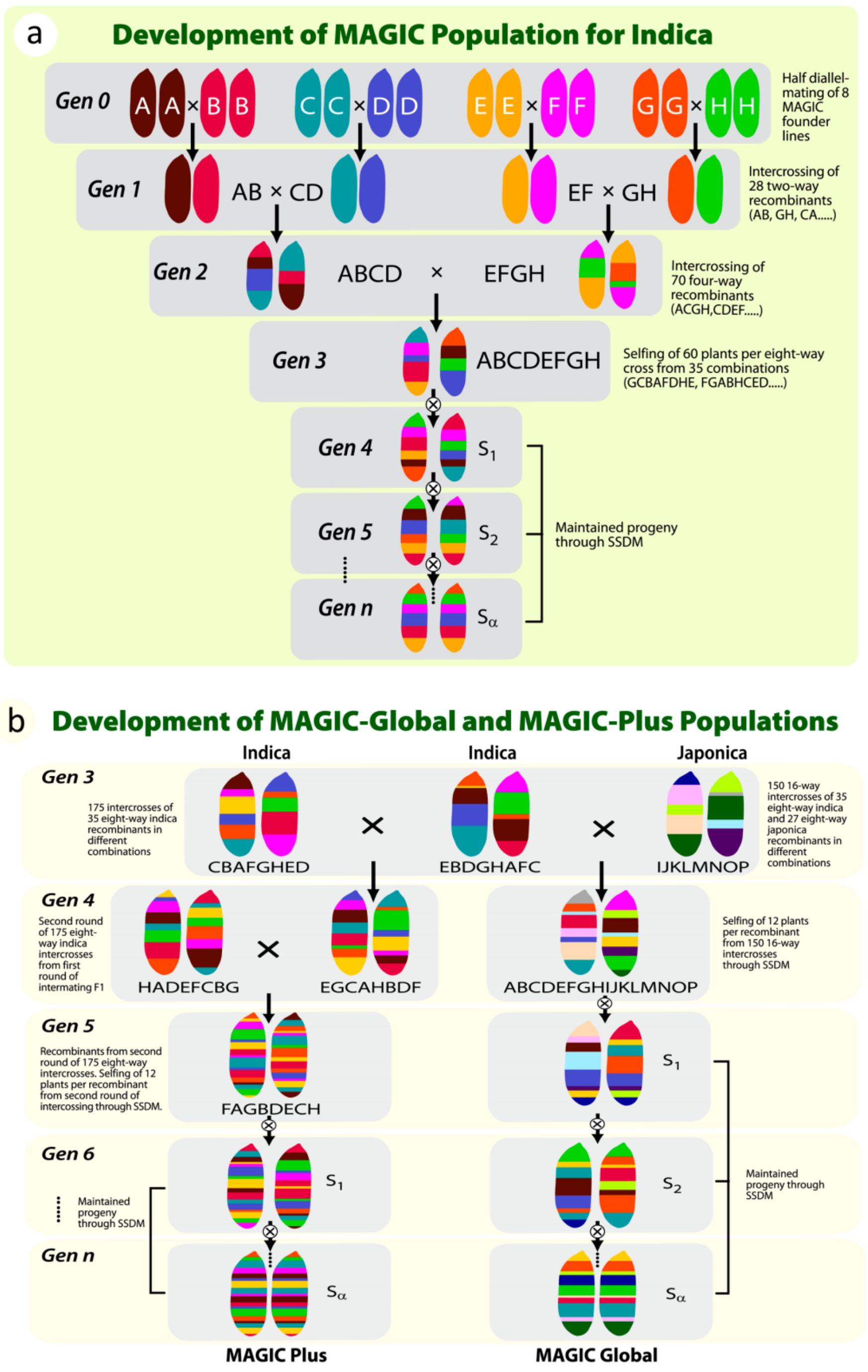
60 seeds from each 8-way was advanced by selfing

1322 S6:8 bulks 2013

Identical scheme was followed to develop the japonica MAGIC population using the 8 japonica founders

498 SSD at S5stage

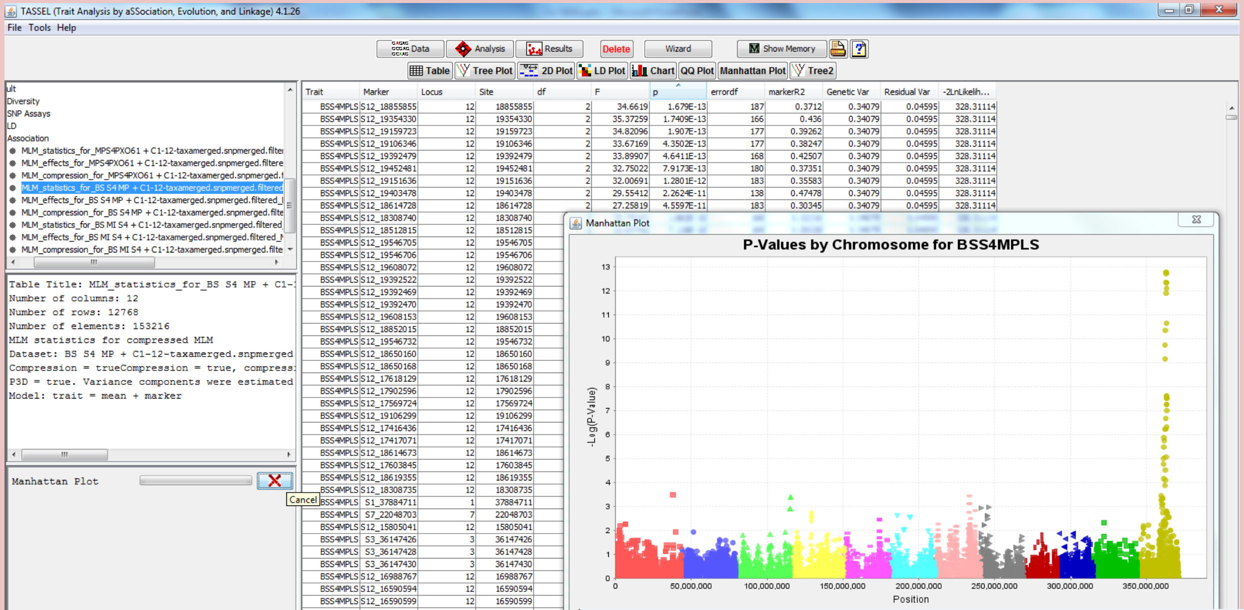
Design of MAGIC Rice



Indica

Japonica

METHODS AND RESULTS

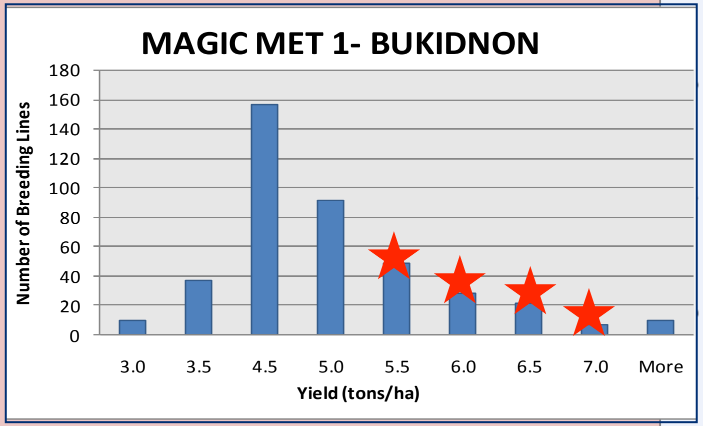


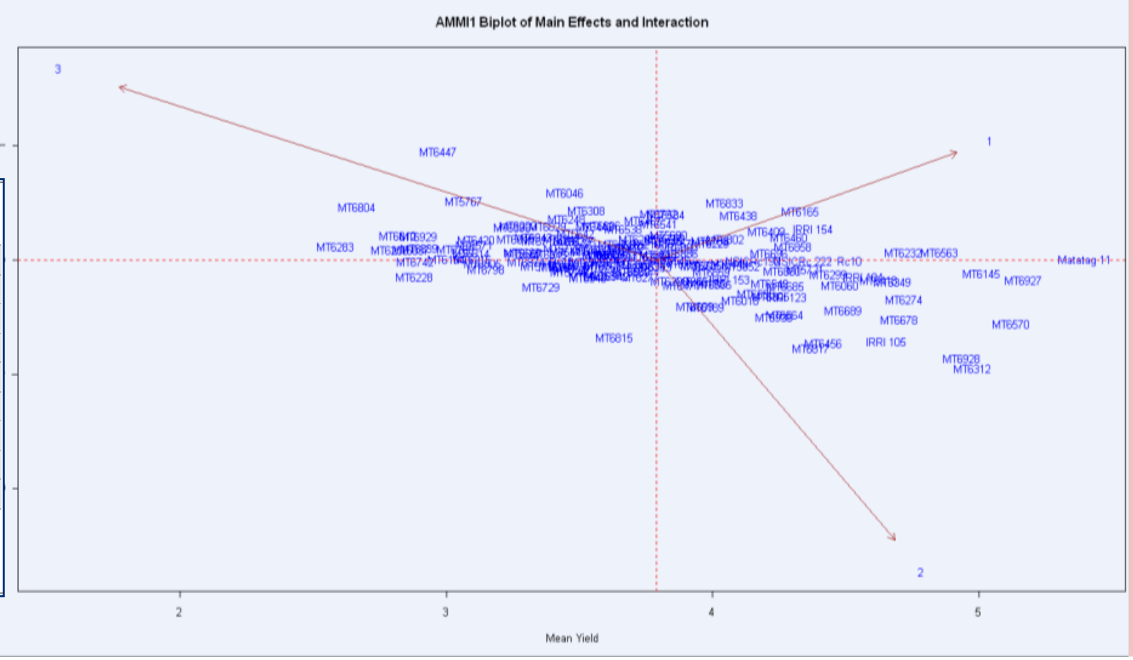
- MLM_statistics_for_MPS4PXO61 + C1-12-taxamerged.snpmerged.filte
- MLM_effects_for_MPS4PXO61 + C1-12-taxamerged.snpmerged.filtere
- MLM_compression_for_MPS4PXO61 + C1-12-taxamerged.snpmerged.f
- MLM_statistics_for_BS S4 MP + C1-12-taxamerged.snpmerged.filtered
- MLM_effects_for_BS S4 MP + C1-12-taxamerged.snpmerged.filtered_I
- MLM_compression_for_BS S4 MP + C1-12-taxamerged.snpmerged.filte
- MLM_statistics_for_BS MI S4 + C1-12-taxamerged.snpmerged.filtered
- MLM_effects_for_BS MI S4 + C1-12-taxamerged.snpmerged.filtered_M
- MLM_compression_for_BS MI S4 + C1-12-taxamerged.snpmerged.filte

```
Table Title: MLM_statistics_for_BS S4 MP + C1-1
Number of columns: 12
Number of rows: 12768
Number of elements: 153216
MLM statistics for compressed MLM
Dataset: BS S4 MP + C1-12-taxamerged.snpmerged
Compression = trueCompression = true, compress
P3D = true. Variance components were estimated
Model: trait = mean + marker
```

```
Manhattan Plot
```

Trait	Marker	Locus	Site	df	F	p	errordf	markerR2	Genetic Var	Residual Var	-2LnLikelih...
BSS4MPLS	S12_18855855	12	18855855	2	34.6619	1.679E-13	187	0.3712	0.34079	0.04595	328.31114
BSS4MPLS	S12_19354330	12	19354330	2	35.37259	1.7409E-13	166	0.436	0.34079	0.04595	328.31114
BSS4MPLS	S12_19159723	12	19159723	2	34.82096	1.907E-13	177	0.39262	0.34079	0.04595	328.31114
BSS4MPLS	S12_19106346	12	19106346	2	33.67169	4.3502E-13	177	0.38247	0.34079	0.04595	328.31114
BSS4MPLS	S12_19392479	12	19392479	2	33.89907	4.6411E-13	168	0.42507	0.34079	0.04595	328.31114
BSS4MPLS	S12_19452481	12	19452481	2	32.75022	7.9173E-13	180	0.37351	0.34079	0.04595	328.31114
BSS4MPLS	S12_19151636	12	19151636	2	32.00691	1.2801E-12	183	0.35583	0.34079	0.04595	328.31114
BSS4MPLS	S12_19403478	12	19403478	2	29.55412	2.2624E-11	138	0.47478	0.34079	0.04595	328.31114
BSS4MPLS	S12_18614728	12	18614728	2	27.25819	4.5597E-11	183	0.30345	0.34079	0.04595	328.31114
BSS4MPLS	S12_18308740	12	18308740								
BSS4MPLS	S12_18512815	12	18512815								
BSS4MPLS	S12_19546705	12	19546705								
BSS4MPLS	S12_19546706	12	19546706								
BSS4MPLS	S12_19608072	12	19608072								
BSS4MPLS	S12_19392522	12	19392522								
BSS4MPLS	S12_19392469	12	19392469								
BSS4MPLS	S12_19392470	12	19392470								
BSS4MPLS	S12_19608153	12	19608153								
BSS4MPLS	S12_18852015	12	18852015								
BSS4MPLS	S12_19546732	12	19546732								
BSS4MPLS	S12_18650160	12	18650160								
BSS4MPLS	S12_18650168	12	18650168								
BSS4MPLS	S12_17618129	12	17618129								
BSS4MPLS	S12_17902596	12	17902596								
BSS4MPLS	S12_17569724	12	17569724								
BSS4MPLS	S12_19106299	12	19106299								
BSS4MPLS	S12_17416436	12	17416436								
BSS4MPLS	S12_17417071	12	17417071								
BSS4MPLS	S12_18614673	12	18614673								
BSS4MPLS	S12_17603845	12	17603845								
BSS4MPLS	S12_18619355	12	18619355								
BSS4MPLS	S12_18308735	12	18308735								
BSS4MPLS	S1_37884711	1	37884711								
BSS4MPLS	S7_22048703	7	22048703								
BSS4MPLS	S12_15805041	12	15805041								
BSS4MPLS	S3_36147426	3	36147426								
BSS4MPLS	S3_36147428	3	36147428								
BSS4MPLS	S3_36147430	3	36147430								
BSS4MPLS	S12_16988767	12	16988767								
BSS4MPLS	S12_16590594	12	16590594								
BSS4MPLS	S12_16590599	12	16590599								
BSS4MPLS	S12_15790736	12	15790736								
BSS4MPLS	S7_22077382	7	22077382								
BSS4MPLS	S7_22077392	7	22077392								
BSS4MPLS	S12_16591814	12	16591814								

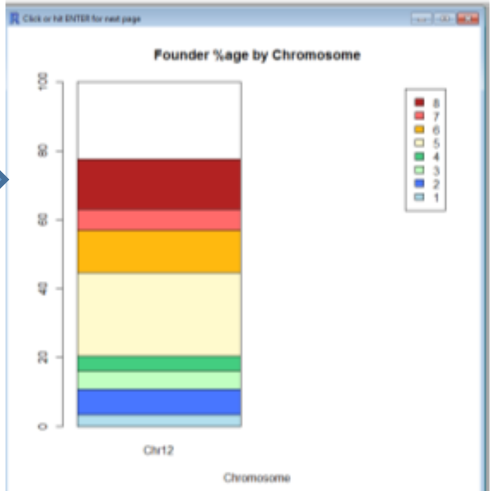




Brown spot disease

Answering the many questions???

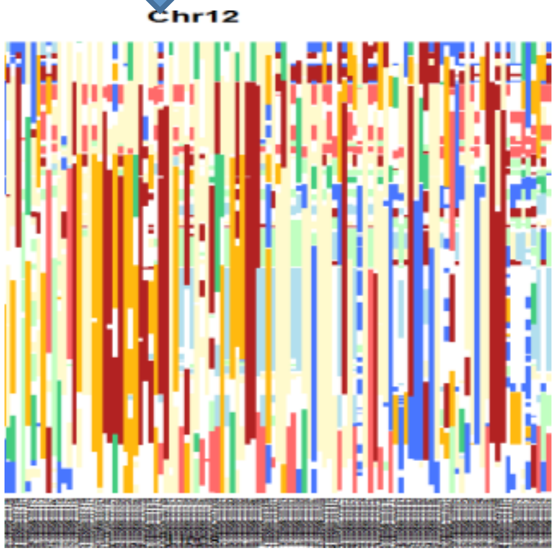
estimate founder representation in the QTL region



Are the founders represented in the population? - Yes

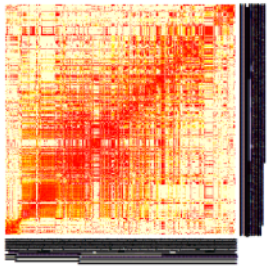
LD

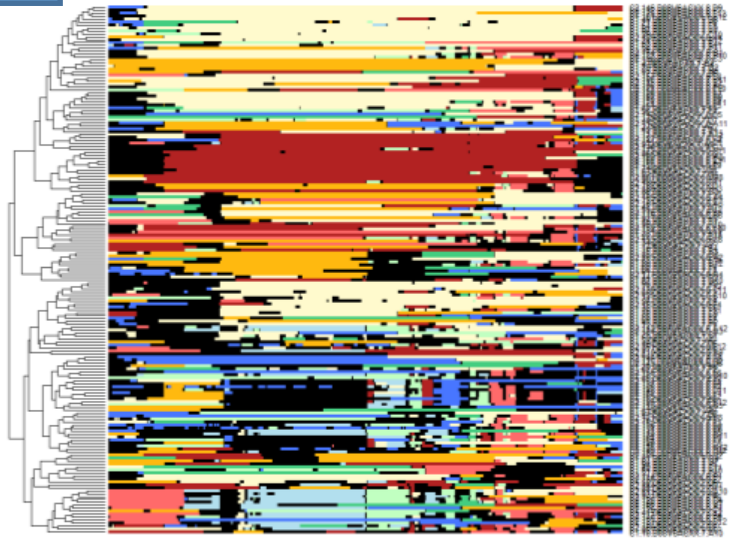
estimate founder probabilities in the QTL region



Lines

Markers





Lines

Work with Dr. Emma Huang