

**Drought tolerant rice cultivars for
North China and South/Southeast
Asia by highly efficient pyramiding
of QTLs from diverse origins**

(GCP Competitive Project # 12)

Zhi-Kang Li

G. Atlin

Institute of Crop Sciences

Chinese Academy of Agricultural Sciences

International Rice Research Institute

Research Team

CAAS

Z.K. Li **Z.T. Hua (LAAS)**
Y.M. Gao **Z.J. Xu (SAU)**
B.Y. Fu **X.B. Hao (LAAS)**
J. L. Xu **J. Niu (TAAS)**
L.H. Zhu

IRRI

G. Atlin and his team
R. Lafitte
D. Mackill
D. Dwivedi (PDF)
J. Domingo (AS)

Students

T.Q. Zheng (PhD) **H. Wang (PhD)**
Y.Z. Jiang (PhD) **Y.J. Pan (PhD)**
S.H. Liu (PhD)
L. Kang (PhD)
Y.X. He (PhD)
L.F. Wang (MS)

Subjects

- **Background**
- **Proof of the concept**
- **Current status and progress**

Background

Two Questions:

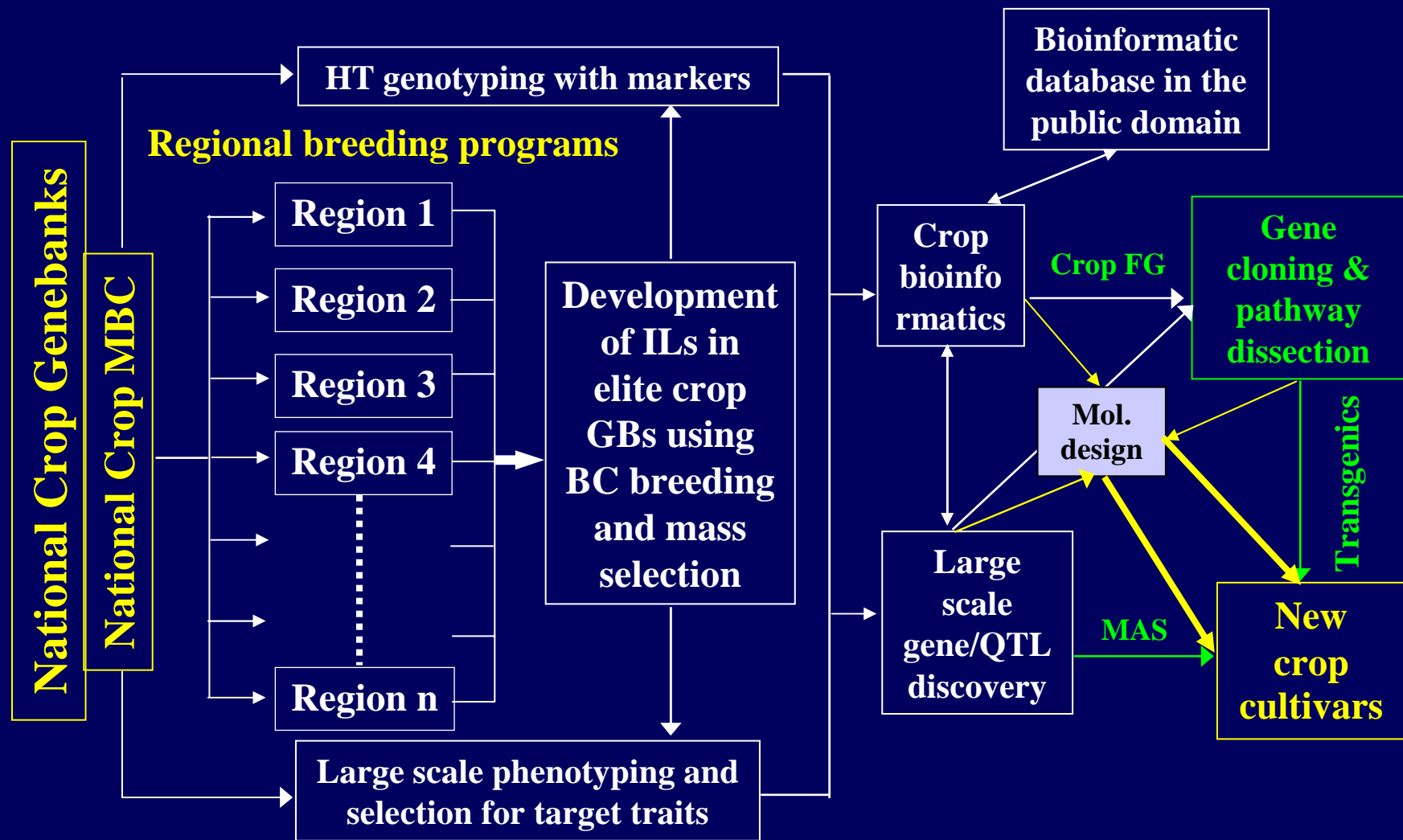
- **How much useful genetic diversity, particular for the complex phenotypes, within the primary gene pools of crops?**
- **Can we combine the process of gene discovery (mapping and cloning) with breeding?**

Initiation of China National Rice Molecular Breeding Program in 1998

Goal

- **Highly efficiently develop superior crop cultivars with greatly improved yield potential and stability for major target environments ;**
- **Discover genes/alleles responsible for important target traits of major crops (rice, maize, wheat and soybean) ;**
- **Establish the material, information, and technological platforms for the national crop molecular breeding programs.**

Organization and Functional Map of the NCMBN



The China National Rice Molecular Breeding Network

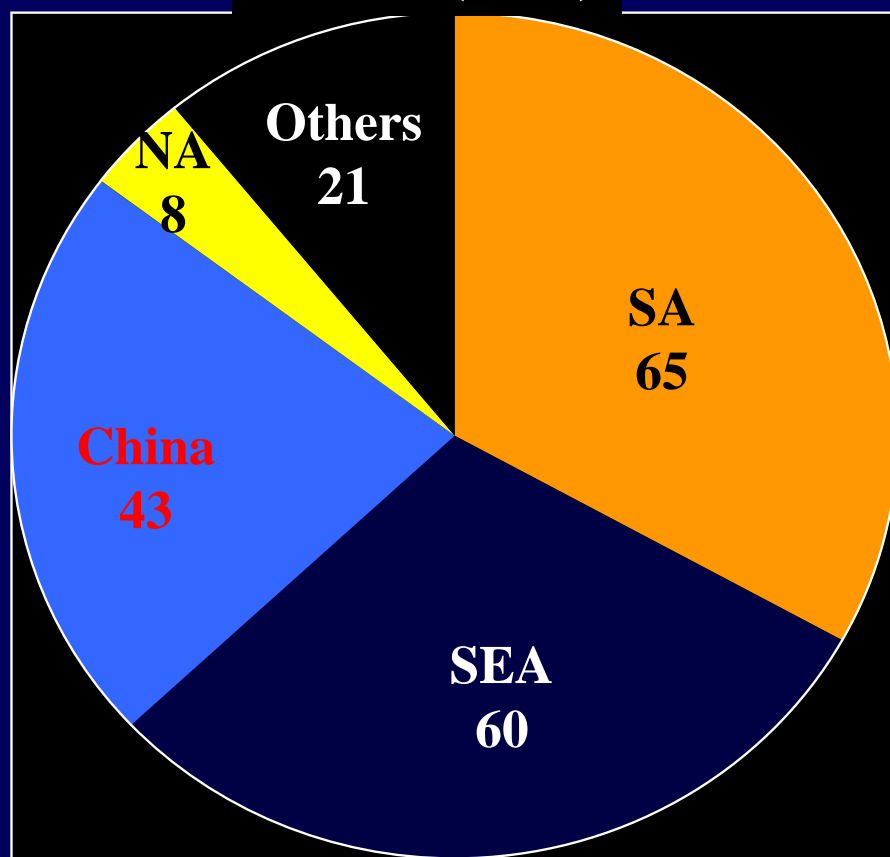


Introgression line sets in 24 elite Chinese rice genetic backgrounds constructed (16 indicas and 8 japonicas)

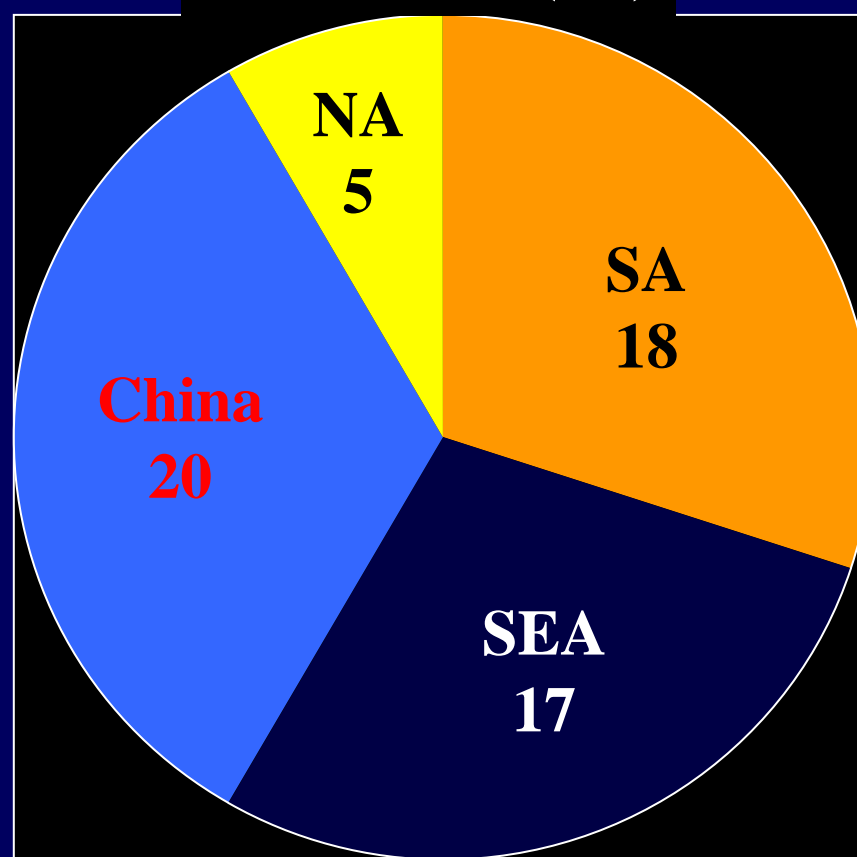
Institutions	Recurrent Parents
IRRI	Teqing(I), NPT(J), IR64(I)
CAAS	Ming-Hui 86(I), Shu-Hui 527(I), Chao-You 1(J)
Huazhong AU	Zhengshan 97(I) 、 9311(I)
SABGC	Zhong 413(I) 、 Hanfeng (J)
Sichuan AAS	Chenghui 448(I) 、 Chuanxiang 29B(I)
CNRRI	Zhong-You-Zhao 81(I)
Shengyang AU	Liaojing 454 (J)
Guandong AAS	Feng-Ai-Zan(I) 、 Yu-Xiang-Zan(I)
Liaoning AAS	C418 (J)
Yunnan AAS	Yun-Hui 290(J) 、 Dian-Tun 502(J) 、 Dian-Xi 4(J)
Anhui AAS	Zhao-Xian 14(I) 、 M3122(J) 、 Zi-Hui 100(I)
Jiangsi AAS	Hui 752(I) 、 Wan-Xian 923(I)

A diverse set of 198 donors from 34 countries (a sample of the mini-core collection of the primary gene pool of rice)

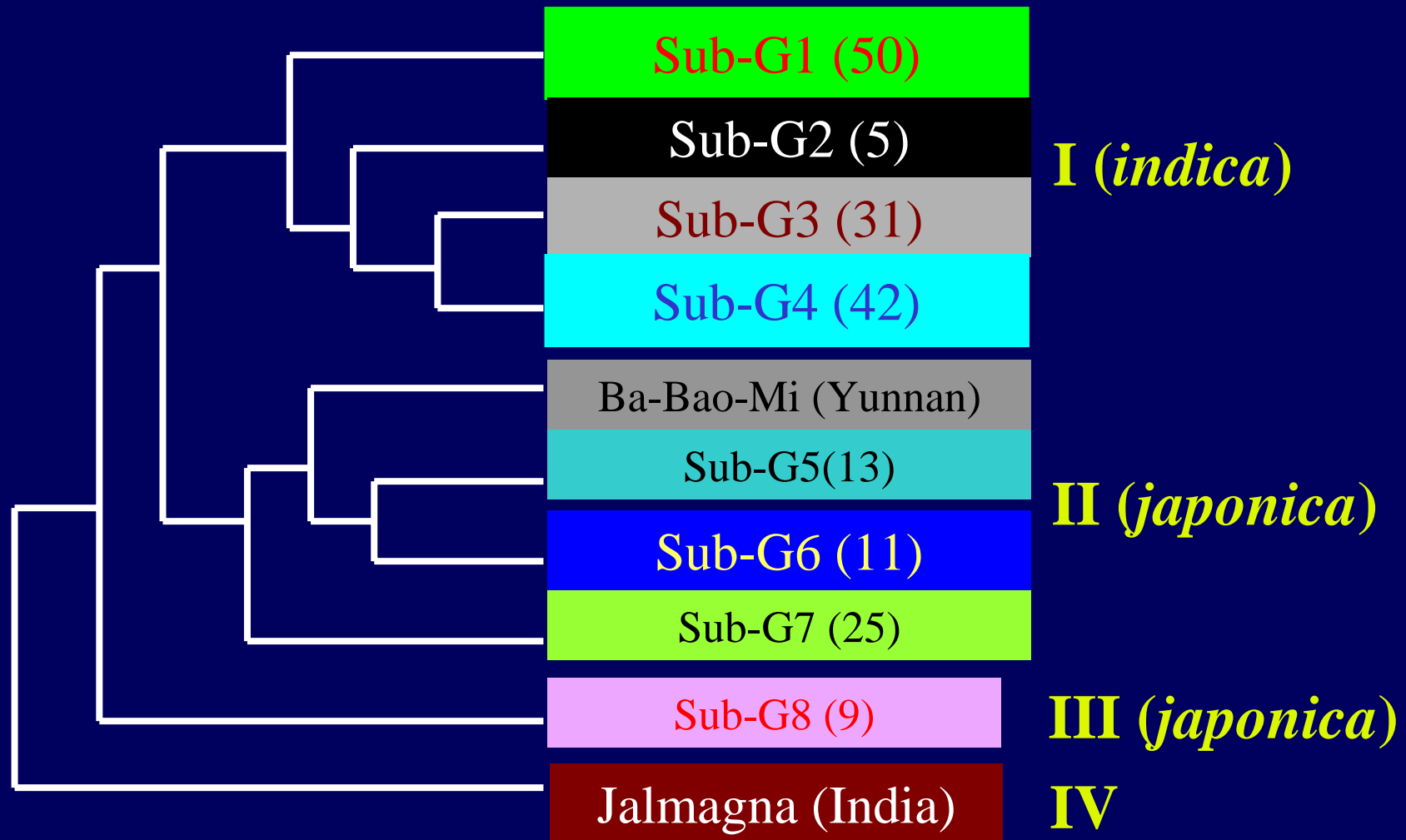
Total (198)



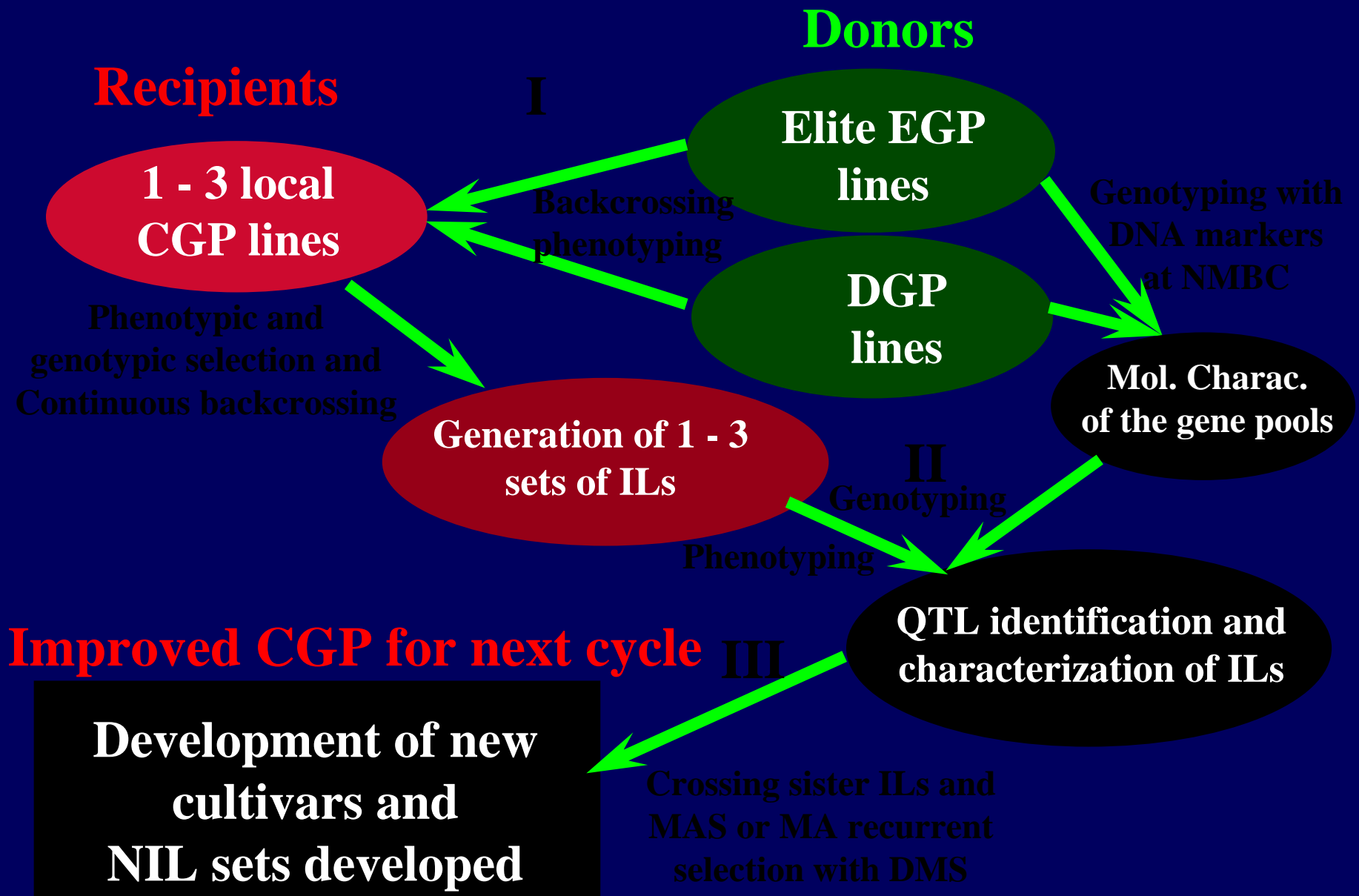
Elite lines (60)



A set of core (198) germplasm accessions from 34 countries used as donors, representing 4 major cultivar groups and 8 subgroups based on 101 SSR markers



Principles and Procedures



China Rice Molecular Breeding Network

'948' Project of MOA

**In 2005, the annual meeting of CRMBN as part of the IRMBN
Coordinated by IRRI – after 7 years of work, we have developed huge
numbers of ILs in 27 elite Chinese rice genetic backgrounds, discovered
many important genes/QTLs, and established the foundation for rice
molecular breeding based on large scale QTL-pyramiding**

Major Progresses

1. More than 3000 crosses have been made between 27 elite lines and 198 donors, and advanced to BC2 – BC3 ;
2. Over 40,000 BC3 bulk populations in 27 elite Chinese GBs developed and stored in SAGIC;
3. More than 1500 BC populations were screened for different target traits and more than 10,000 ILs developed ;
4. More than 20 important QTLs/alleles discovered and mapped for important traits ;
5. 12 new cultivars developed, 5 of which have passed the multilocational trials, and a large number of promising lines are in the pipeline ;
6. More than 20 publications.

The Definition of the National Crop Molecular Breeding Networks (NCMBN)

NCMBN can be considered as the national crop breeding networks and technical/material platforms. In this platform, large scale BC breeding activities, deep exploitation of useful genetic diversity, accurate selection for target traits, marker-based gene-flow tracking, and large-scale gene/QTL and allelic diversity discovery, accumulation of the relevant genetic information of target traits, and information based trait design and marker-aided selection are fully integrated with the conventional breeding process such that superior crop cultivars with greatly improved yield potential, stability and quality for major target environments can be developed in a highly efficient way.

The Functions of NCMBN (1)

1. National Center (CAAS or Key Agricultural Universities) :

- **Selection and information (genotypic and phenotypic) of the parents from the core germplasm collections ;**
- **Selection and coordination of testing sites for screening target traits ;**
- **BC breeding and IL development for a specific TE ;**
- **IL genotyping and data analyses for gene/QTL discovery and allelic mining ;**
- **Storage and maintenance of the parents and developed ILs and relevant information and database ;**
- **Theory and methodology for development of cultivars by genetic/molecular designing and MAS;**
- **Annual meetings for MBN ;**
- **Advanced training for plant molecular breeders.**

The Functions of NCMBN (2)

1. Participants (Provincial Agr. Academies and Universities) :

- **BC breeding and IL development for a specific TE ;**
- **IL phenotyping for gene/QTL discovery/allelic mining ;**
- **Storage and maintenance of the locally developed ILs and relevant information and database;**
- **Execute MAS ;**
- **Extension of the developed Cultivars.**

Proof of the Concept

Part I

BC Breeding and Selection Experiments

Old techniques – BC breeding and mass selection

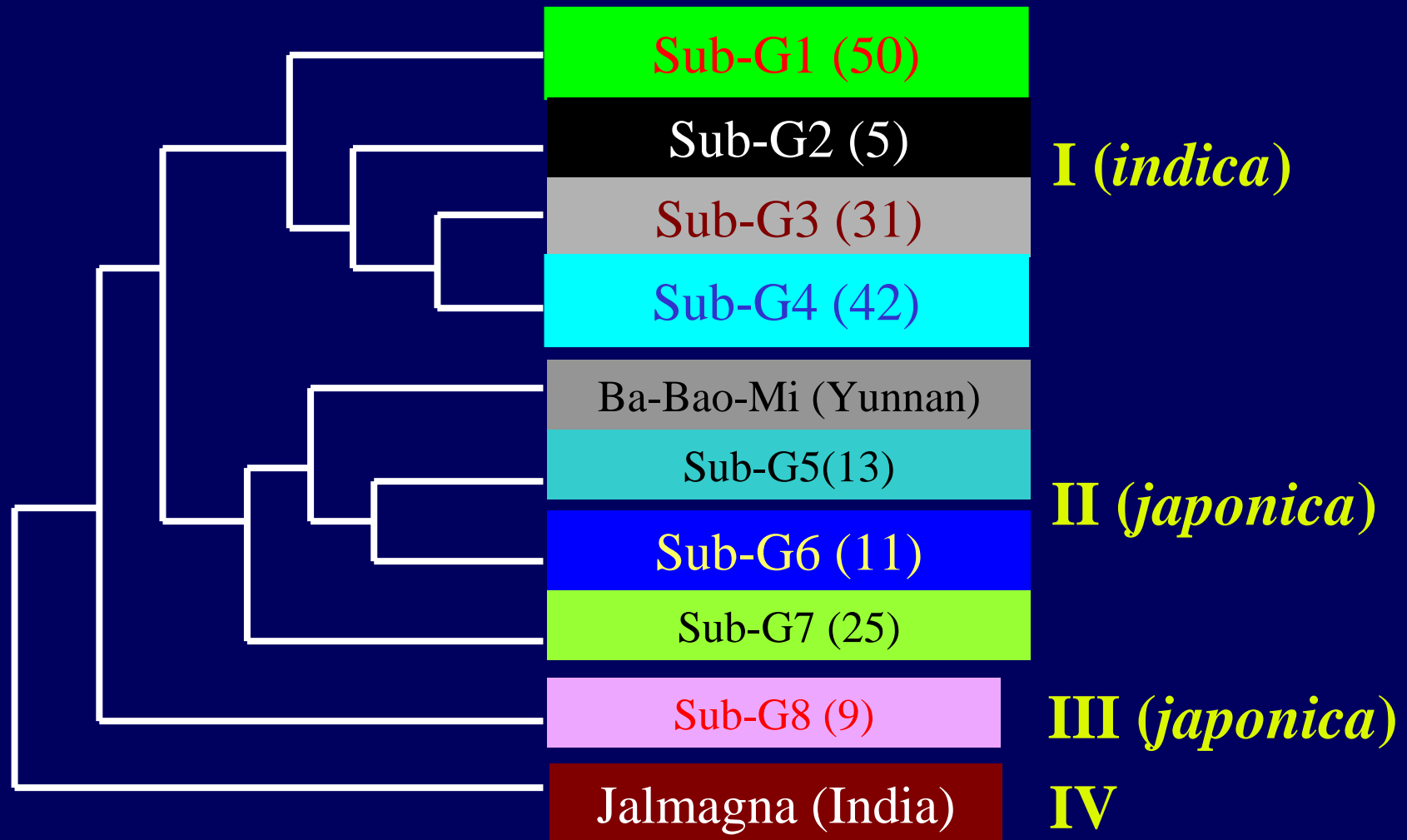
Recurrent Parents

IR64 - Indica, high yield/widely adaptable

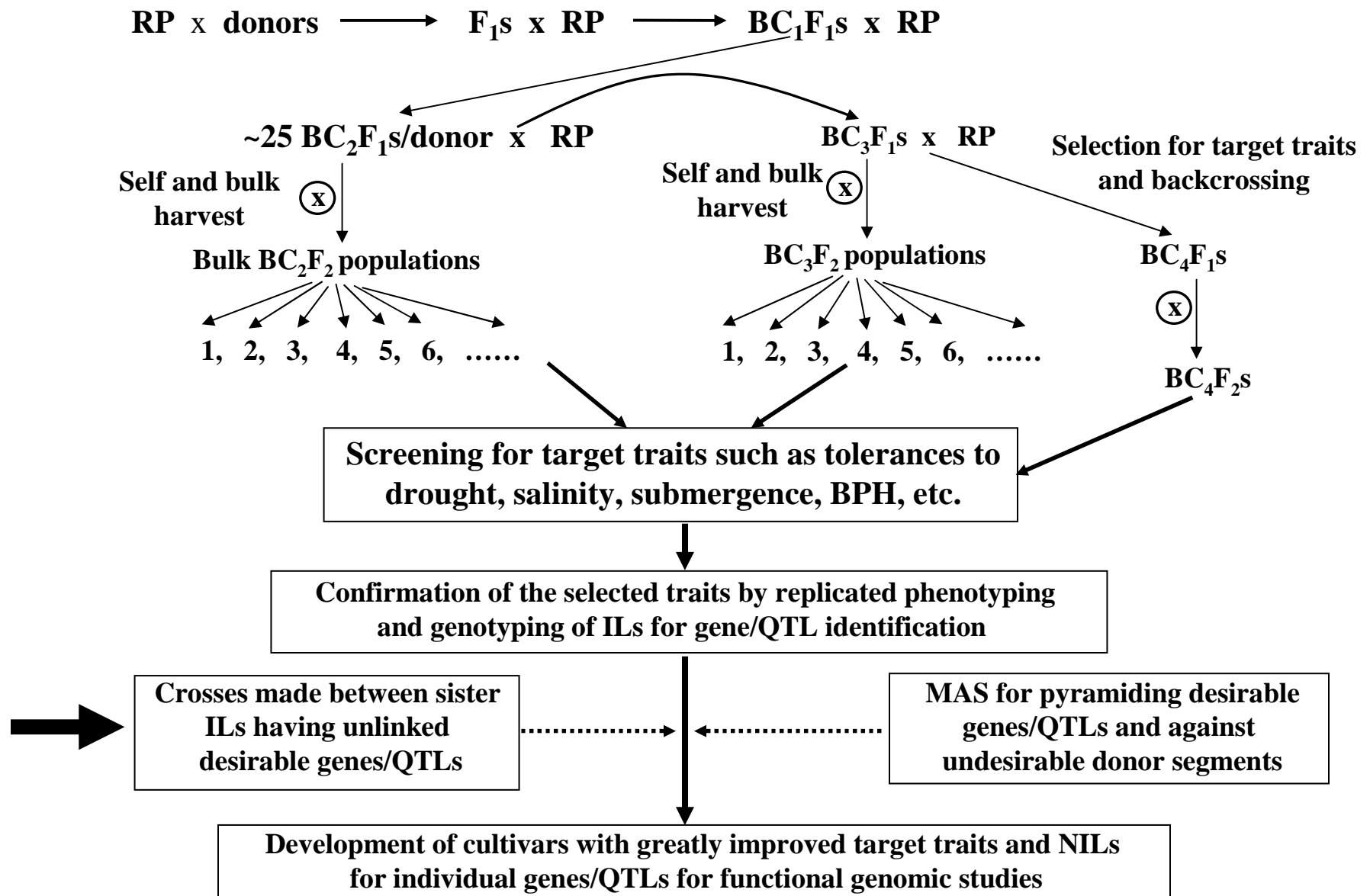
New Plant Type - Japonica, high yield potential

Teqing - Indica, high yield/widely adaptable

A set of core (198) germplasm accessions from 34 countries used as donors, representing 4 major cultivar groups and 8 subgroups based on 101 SSR markers



Procedure of the backcross breeding for development of ILs for gene/QTL identification and MAS for cultivar development



**Screening and variation of BC₂F₂
populations for drought tolerance under
the lowland conditions**

Screening of IR64 BC populations under the upland drought



IR64

Screening of BC₂F₂ populations for submergence tolerance

Thirty-five-day old seedlings were submerged under deep water for two weeks, then water drained and plants were allowed to recover.

Screening of BC₂F₂ populations for salinity tolerance at the seedling stage

Young seedlings were subjected: 6 dSm⁻¹ for 3 days, 12 EC dSm⁻¹ for 2 weeks, EC 18 dSm⁻¹ for 1 week, and 24 EC dSm⁻¹ for 1 week

RP

ST CK
Pokali



Screening of BC₂F₂ populations for anaerobic germination under 10 cm deep-water

Screening for tolerance to phosphorus deficiency

Tested in the natural P-deficient conditions of Pangil, Laguna.

Screening for resistance to BPH

Stay green type

Introgression lines developed for different phenotypes

Target traits	# of BC ₂ F ₂ populations	No. of selected BC ₂ F ₃ lines
Drought tolerance	350	4687
BPH resistance	203	522
Salinity tolerance	203	1022
Anaerobic germination	130	368
Zinc deficiency	129	1211
Submergence tolerance	264	798
Grain quality	65	580
Other traits	375	12,000+
Total	1719	20,000+

Summary of Selection Experiments

- Most donors contributed performance enhancing alleles for most traits screened to their BC progenies regardless of their performance;
- BC progeny with extreme phenotypes for complex phenotypes are frequently identified in crosses involving “inferior” parents and appropriate screening (selection) is the key to identify improved target traits in the BC progenies;
- More distantly related donors, particularly landraces, tend to give more transgressive segregations for complex phenotypes in the BC progenies.

Details: see Ali et al. (2005) and Lafitte et al. (2005) in FCR.

Part II

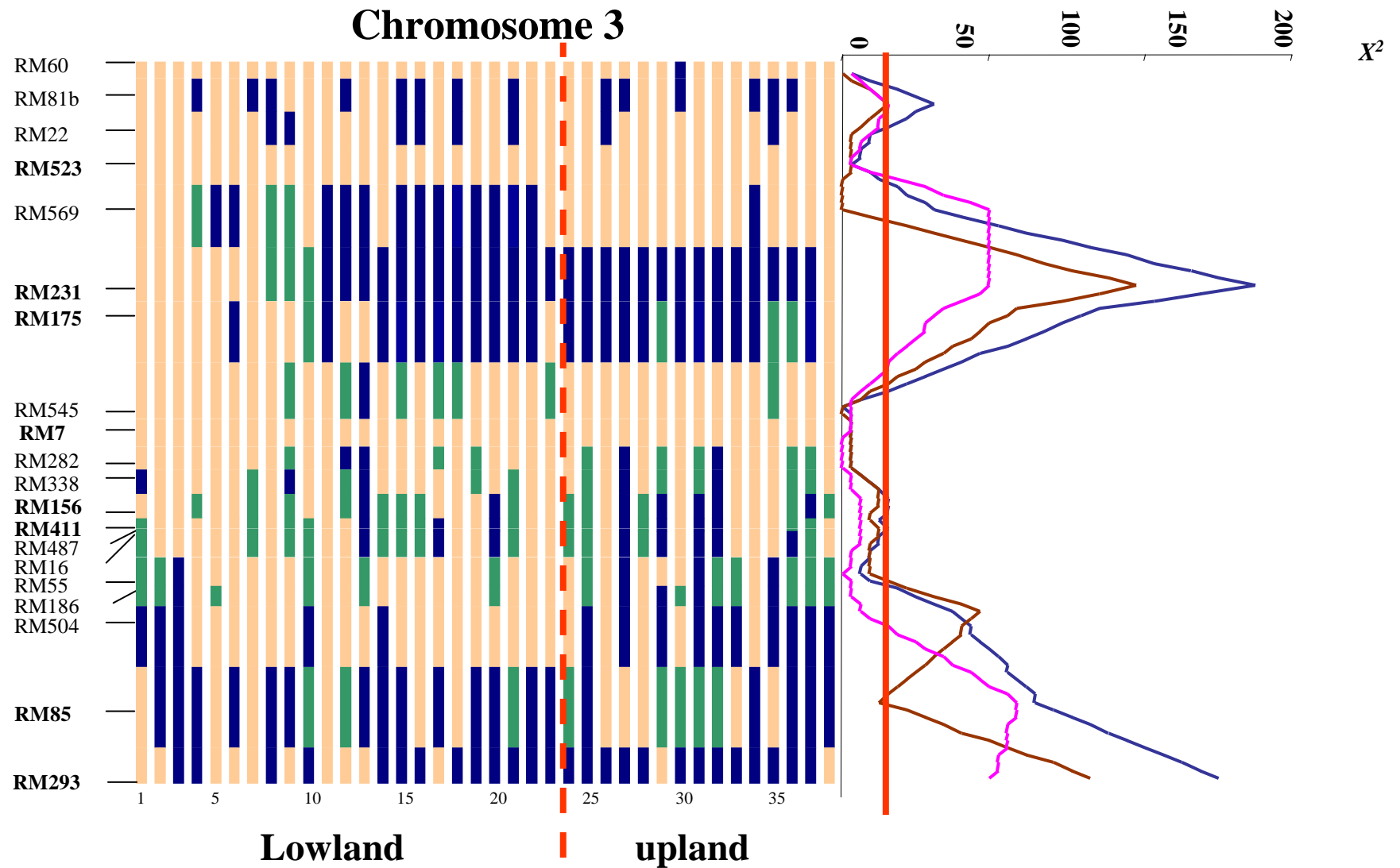
Discover genes/QTLs for target traits using ILs

- **Tracing the gene flow and mining allelic diversity for abiotic tolerances in the selected ILs**
- **Discovering genetic networks underlying abiotic tolerances in rice**

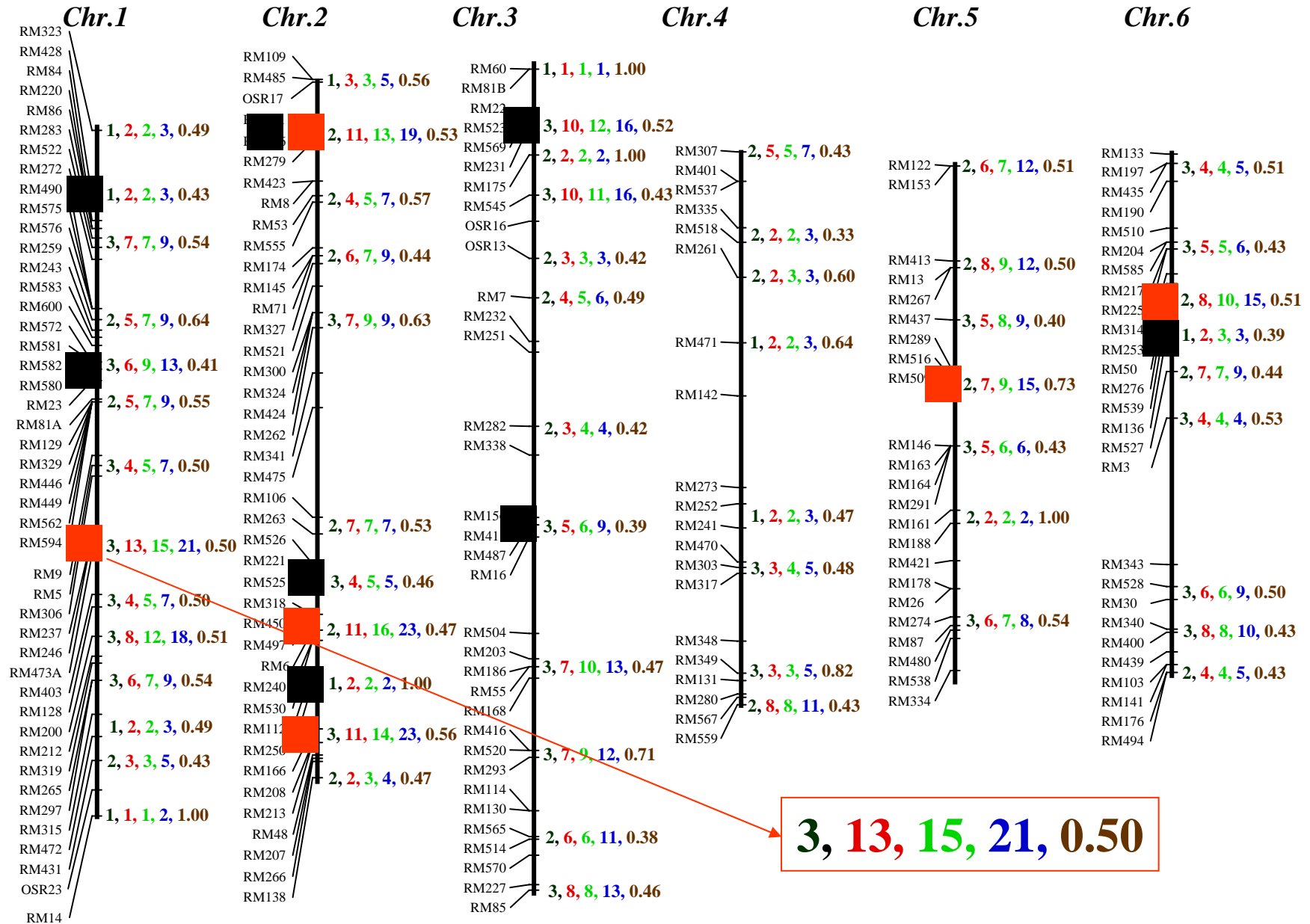
794 DT BC₂F₂ progeny selected from 68 BC populations (13,936 plants) derived from 41 crosses between 3 RPs and 20 donors

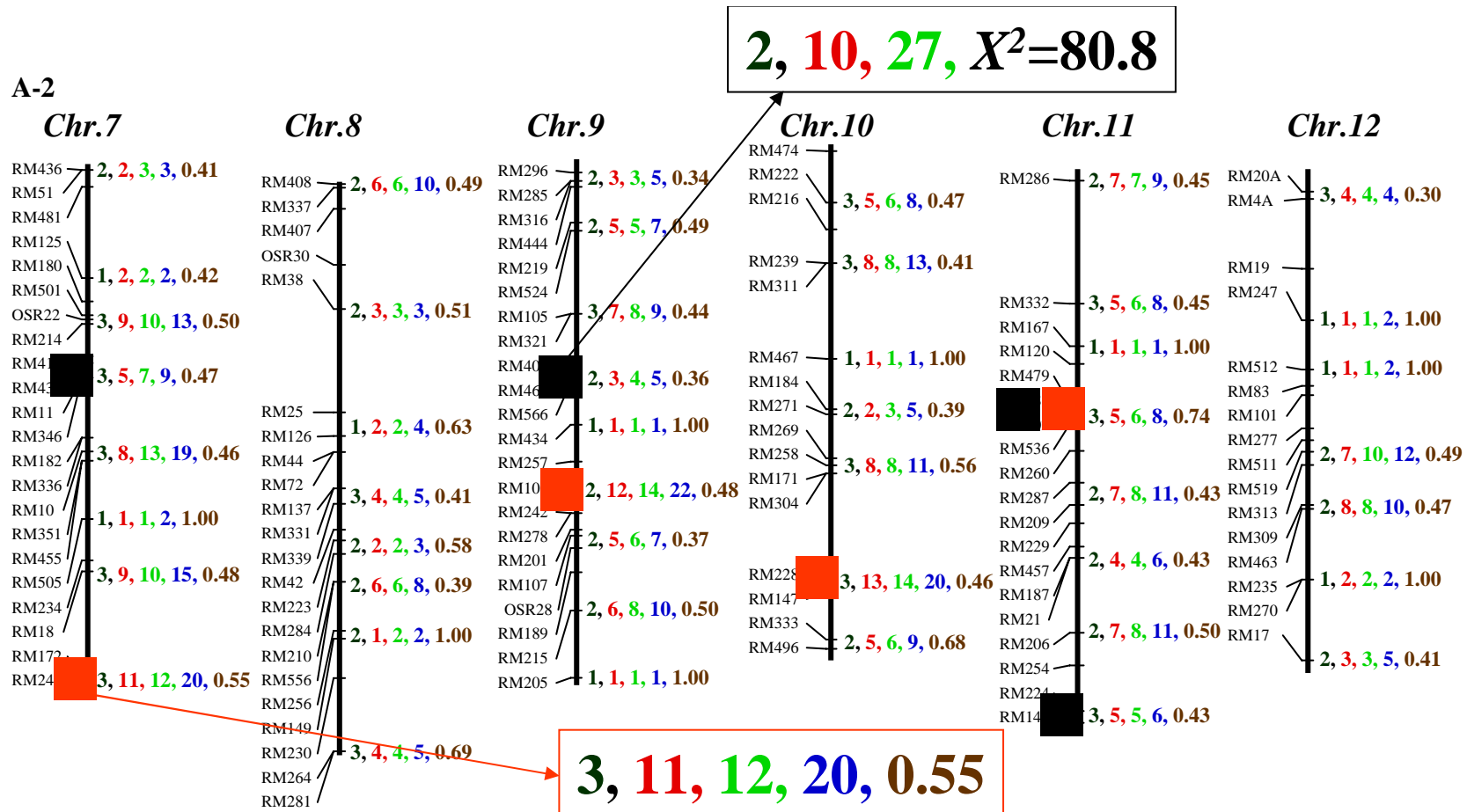
VG	Donors	Origin	IR64 (S) LL (UL)	Teqing (M) LL (UL)	NPT(SS) LL	Total
I	BR24 (I)	Bangladesh	14 (27)	12 (7)	3	29 (34)
I	STYH (I)	Myanmar	20 (26)	8	4	24 (26)
I	OM1723 (I)	Vietnam	7 (17)	7 (6)		14 (23)
J	FR13A (I)	India	15 (16)	17 (15)		32 (31)
J	Type3 (J)	India	23 (15)	10 (12)		33 (27)
J	Binam (J)	Iran	20 (19)	14 (13)	1	35 (32)
J	HAN (J)	China	11 (13)	-	3	14 (13)
I	Zihui100 (I)	China	8	-	9	17
J	Khazar (J)	Iran	58	-		58
I	Bg300 (I)	Sri Lanka	16 (12)	10 (5)		26 (17)
I	Bg304 (I)	Sri Lanka	6 (12)	4		10 (12)
I	BR11 (I)	Bangladesh	7 (16)	6		13 (16)
I	Chenghui448 (I)	China	8 (12)	9 (3)		17 (15)
I	Babaomi (I)	China	8 (10)	10		18 (10)
I	Basmati (J)	India	16 (7)	12 (10)		28 (17)
I	Lemont (J)	USA	9 (9)	9		18 (9)
I	Cisanggarung (I)	Indonesia	9 (7)	13		22 (7)
I	Tarom Molaii (J)	Iran	8 (19)	8 (3)		16 (22)
I	MR159 (I)	Malaysia	3	15 (6)		18 (6)
I	Yu Qiu Gu (I)	China		12 (14)		12 (14)
	Total		267 (237)	176 (94)	20	463 (331)

Detection of six DT QTLs on chromosome 1 in 38 DT ILs from the IR64/Type3//IR64 BC population and linkage disequilibrium



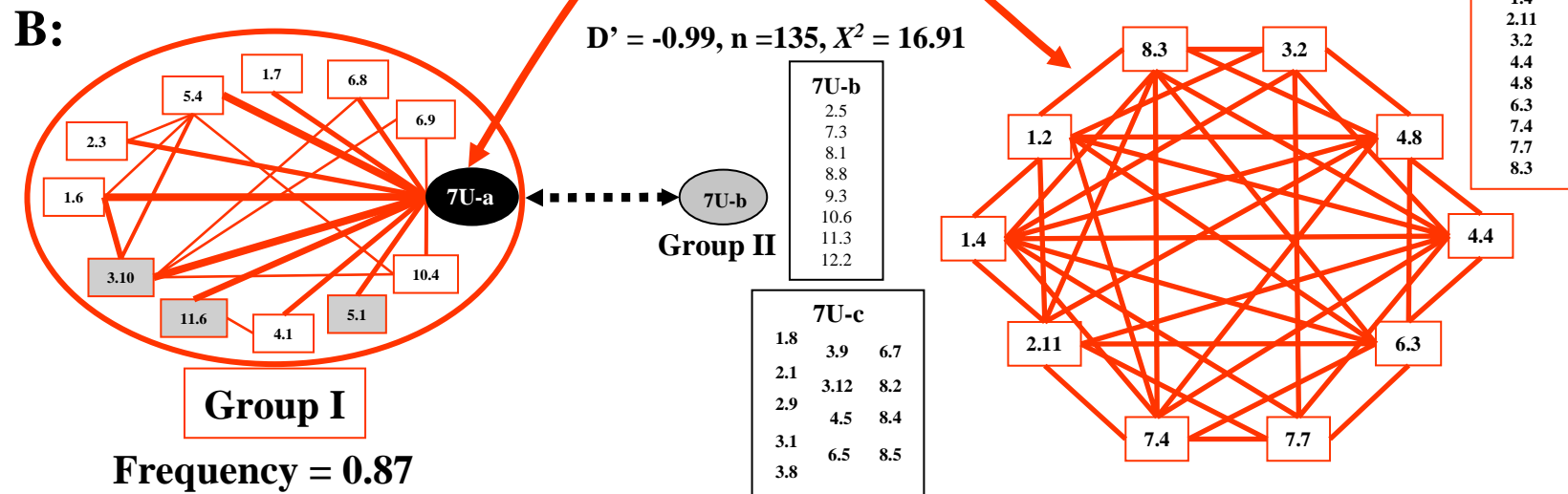
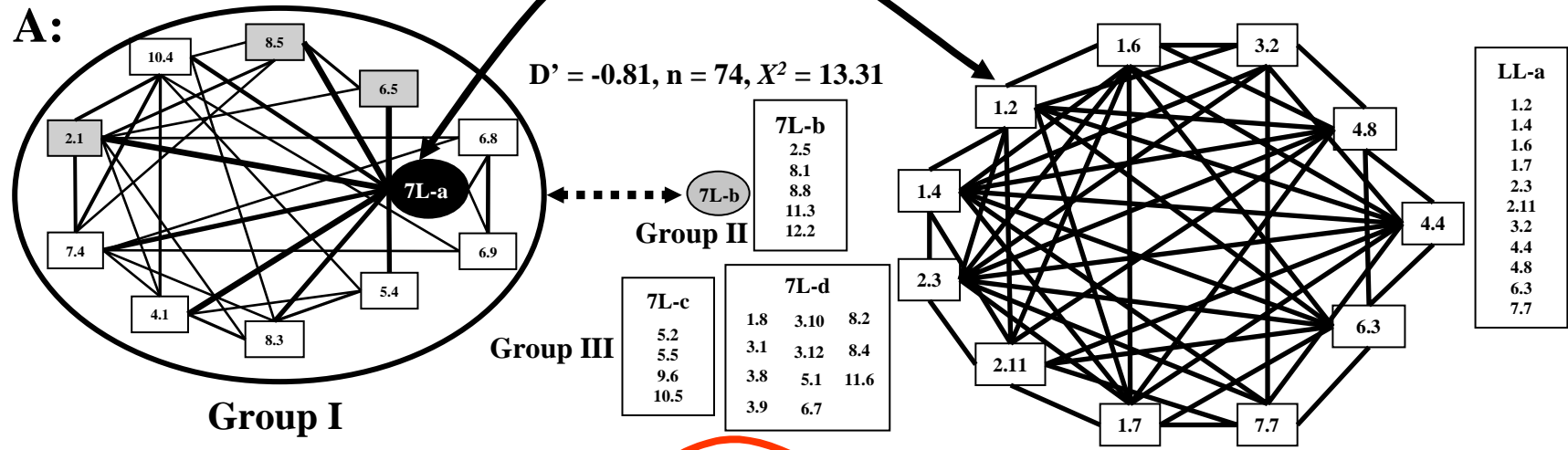
Genomic distribution of 104 bins of excess introgression



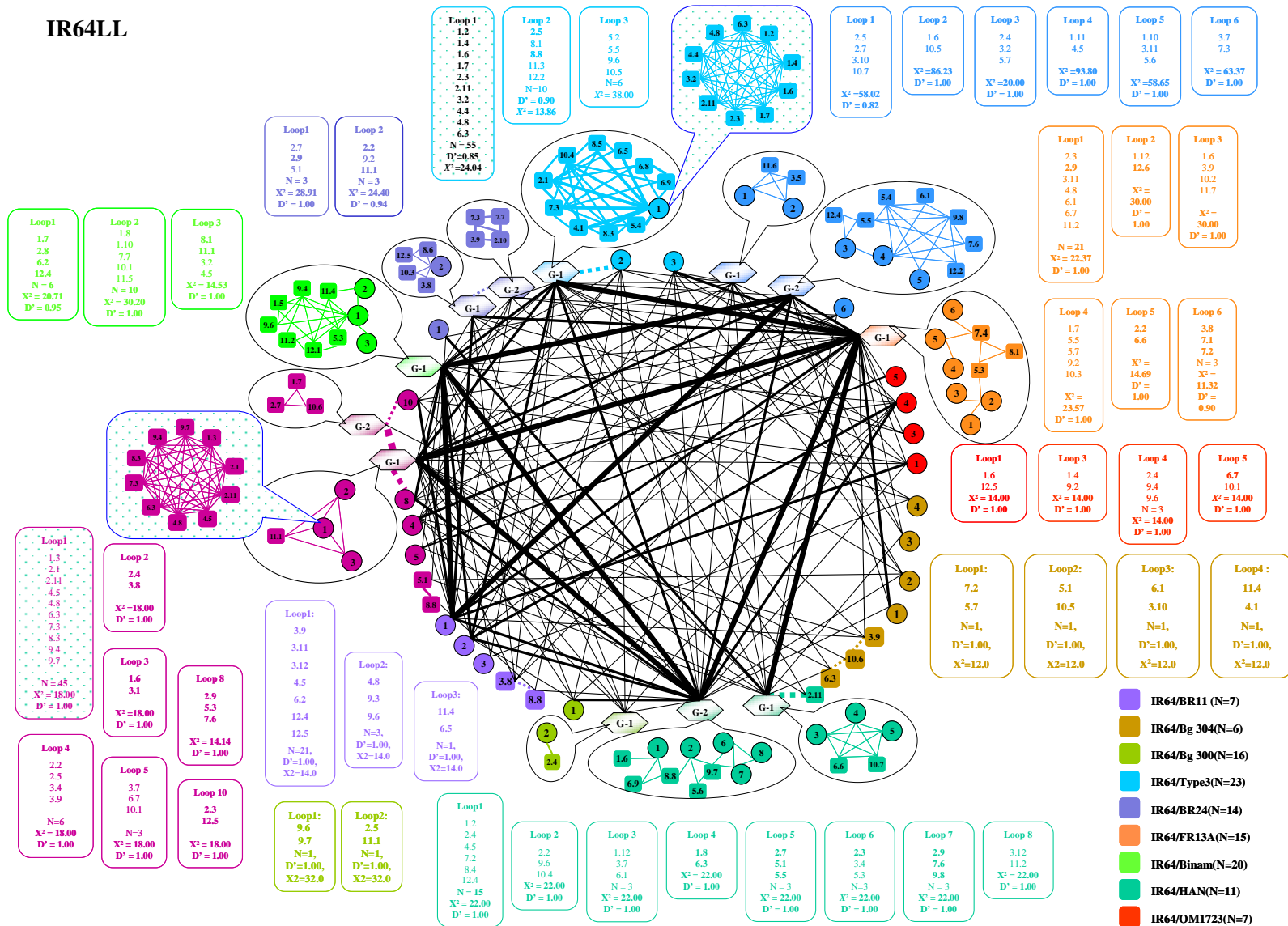


On average, each of the 104 haplotypes was detected in 138 ILs from 7.3 populations involving 2.3 recipients and 6.2 donors with introgression frequency of 0.46 ± 0.20 , or 3.7 times as much as expected. In addition, zero introgression was observed at 80 haplotypes, each detected in 154.5 ILs from 12.7 populations involving 2 RPs and 5.7 donors.

Genetic networks revealed by 244 (249) non-redundant LDs between 30 (29) DT loci in 23 (15) DT IR64/Type3 ILs

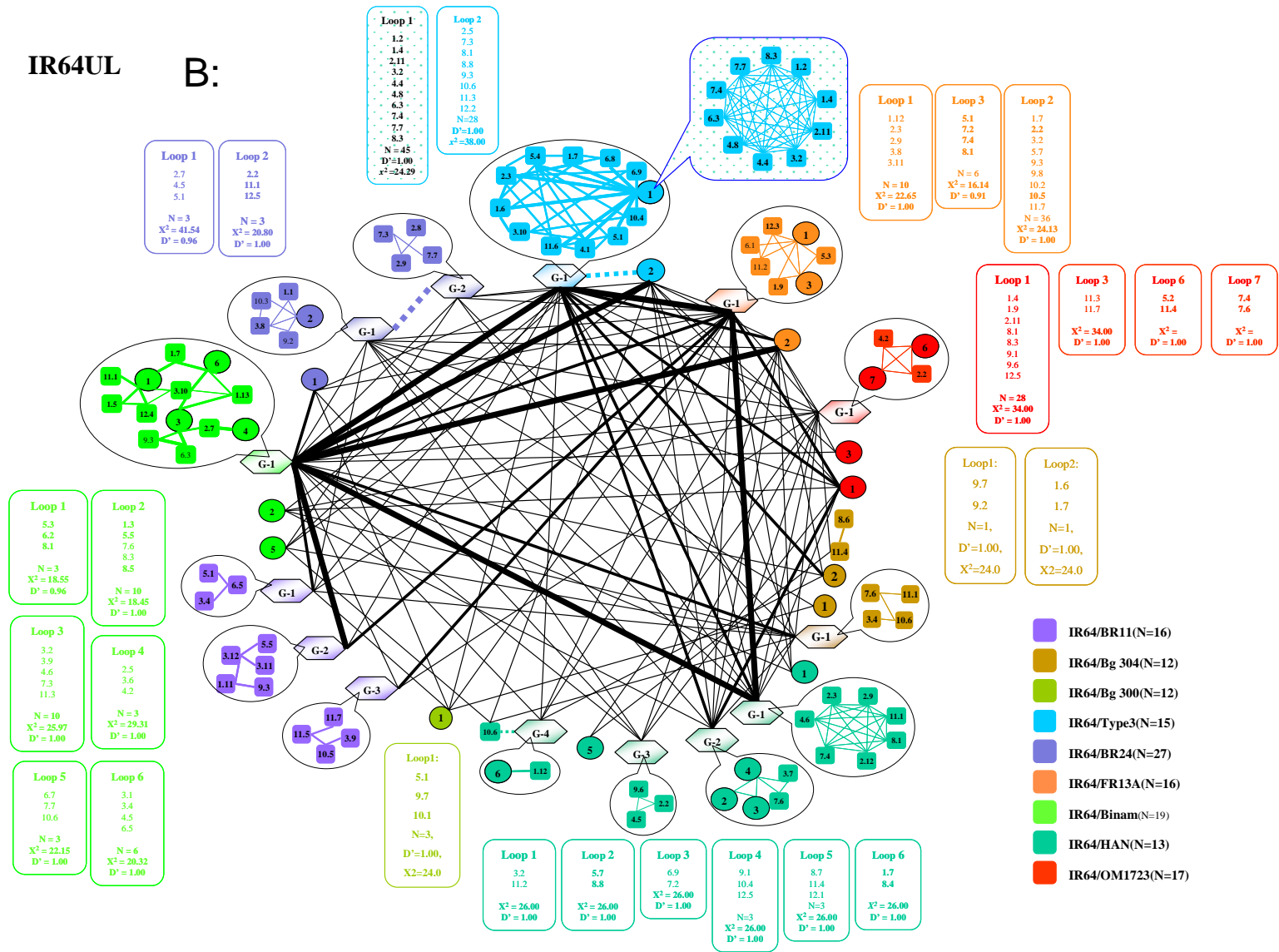


IR64LL



Single Double Triple or more
 102 26 8

IR64UL B:



- IR64/BR11(N=16)
- IR64/Bg 304(N=12)
- IR64/Bg 300(N=12)
- IR64/Type3(N=15)
- IR64/BR24(N=27)
- IR64/FR13A(N=16)
- IR64/Binam(N=19)
- IR64/HAN(N=13)
- IR64/OM1723(N=17)

Single Double Triple or more
81 13 7

**For the methodology of using
selective introgression and DNA
markers for gene/QTL discovery:**

See: Li et al. (2005) PMB

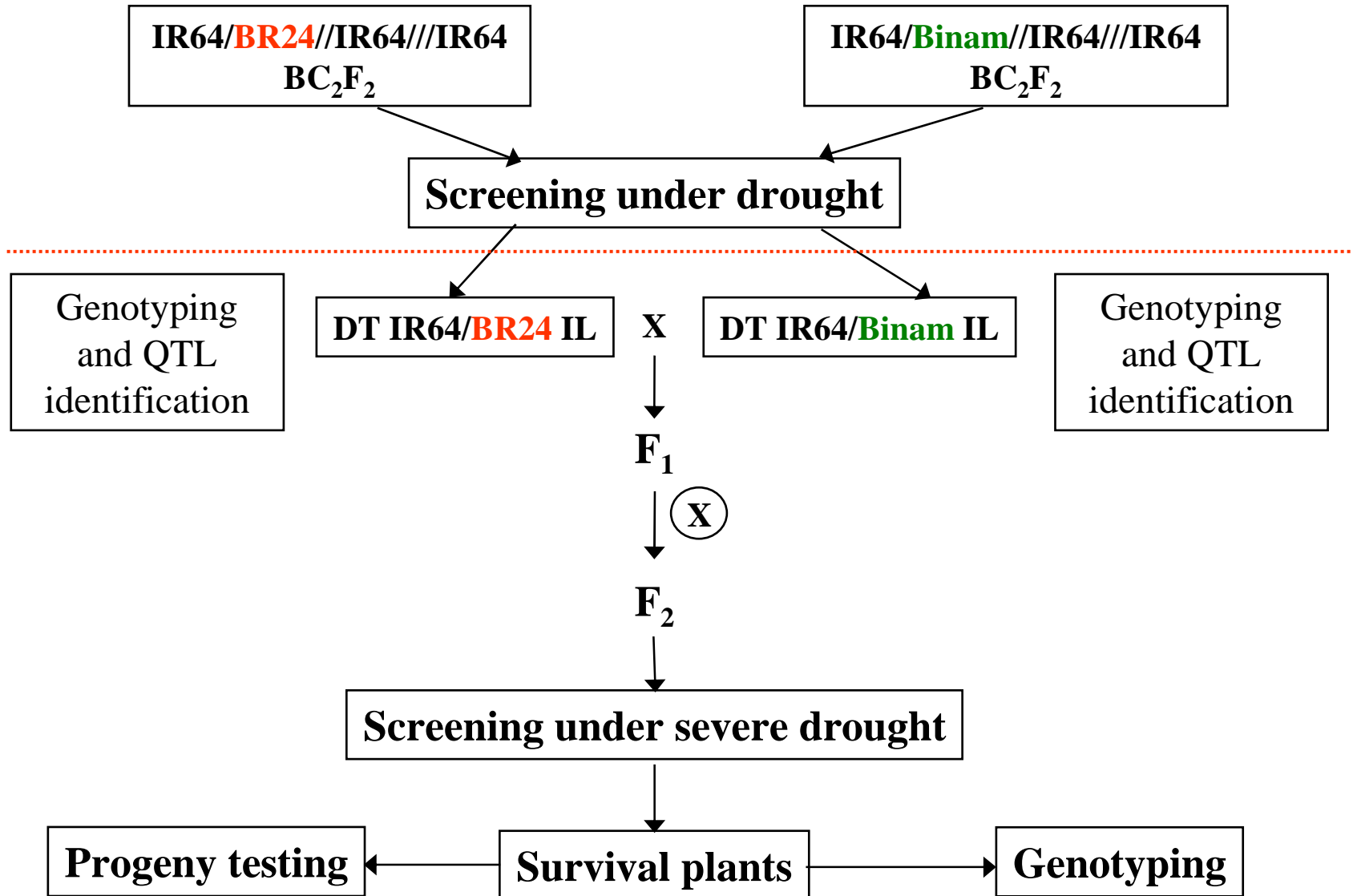
Part III

**Development of DT IR64 lines by QTL
Pyramiding and Verification of the
Genetic Networks of DT in Rice**

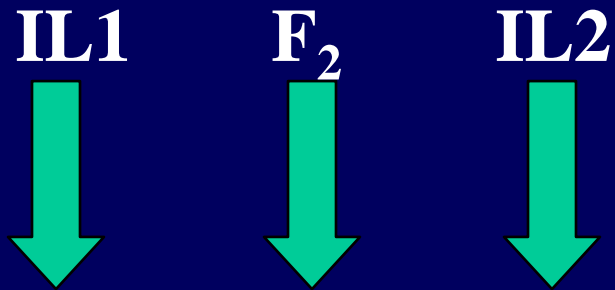
Fourteen parental DT IR64 ILs, 7 donors (from 6 countries), crosses and the number of selected DT F₂ plants

Cross	Female IL		Male IL		F ₂ population	
	Code	Donor	Code	Donor	<i>N</i>	<i>n</i>
1	1	STYH (I)	5	BR24	237	25
2	1	STYH	6	BR24	190	55
3	2	STYH	10	Zihui100	299	30
4	3	BR24 (I)	11	Binam (J)	318	90
5	3	BR24	12	OM1723 (I)	305	105
6	4	BR24	12	OM1723	248	55
7	4	BR24	11	Binam	154	30
8	7	Type3 (J)	13	Haoannong (J)	255	70
9	8	Type3	10	Zihui100	135	70
10	9	Zihui100 (I)	14	Haoannong	219	30
					560 (24.8%)	

Marker aided pyramiding of DT QTLs



Screening of F_2 population (DS 2002-03)



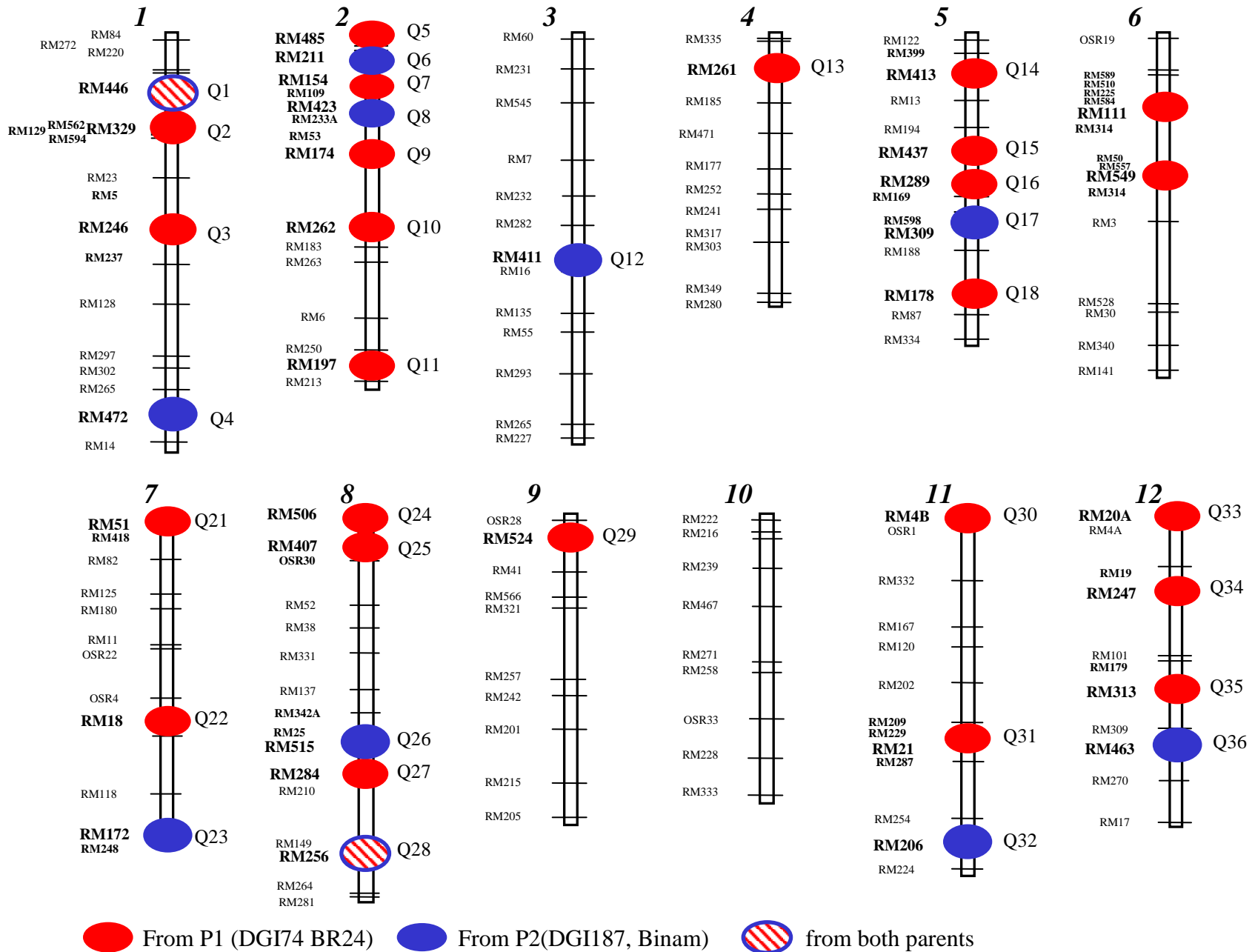
Phenotyping (progeny testing):

- **The selected DT progenies were phenotyped in replicated trails under both stress and controlled situation during DS 2003-04.**
- **Observations made on heading time, plant height, fertility and yield performance.**

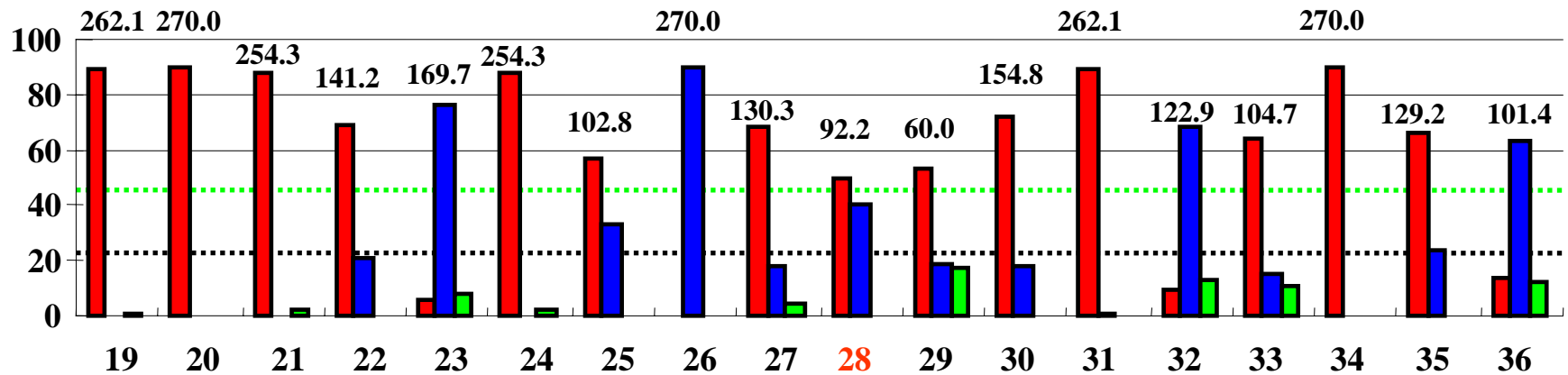
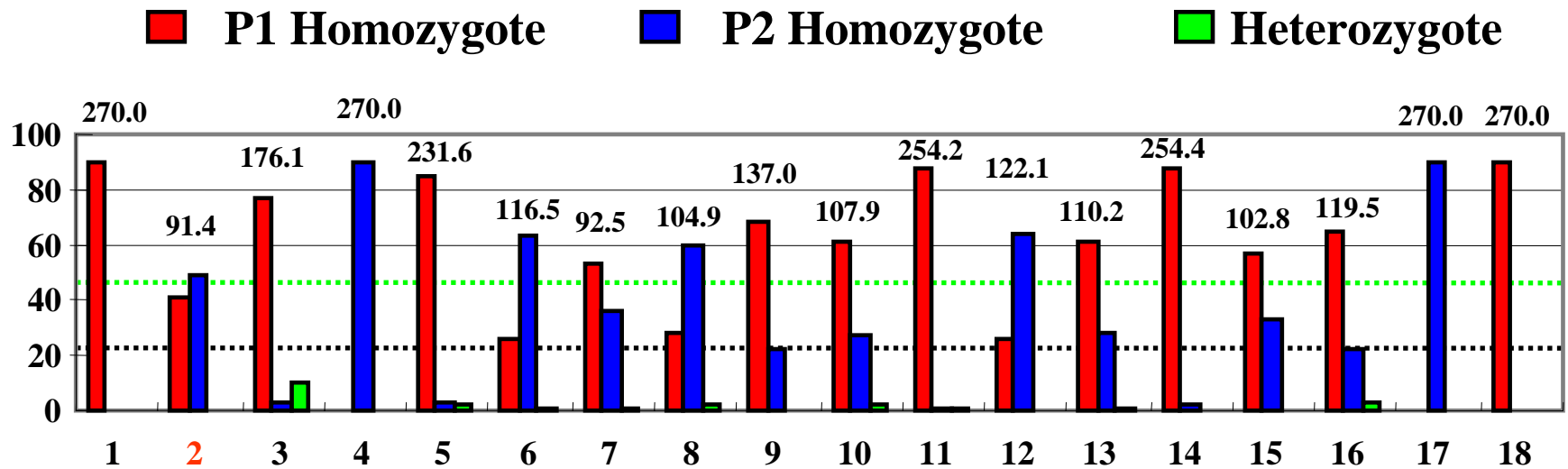
Genotyping were done with differentiating SSR markers

Results

Genomic distribution of the 36 segregating DT QTLs (cross 2)



Selection effects on genotypic frequencies at DT QTLs



Population 2 with 90 selected F₂ individuals derived from a cross between two ILs (IR64/BR24 x IR64/Binam)

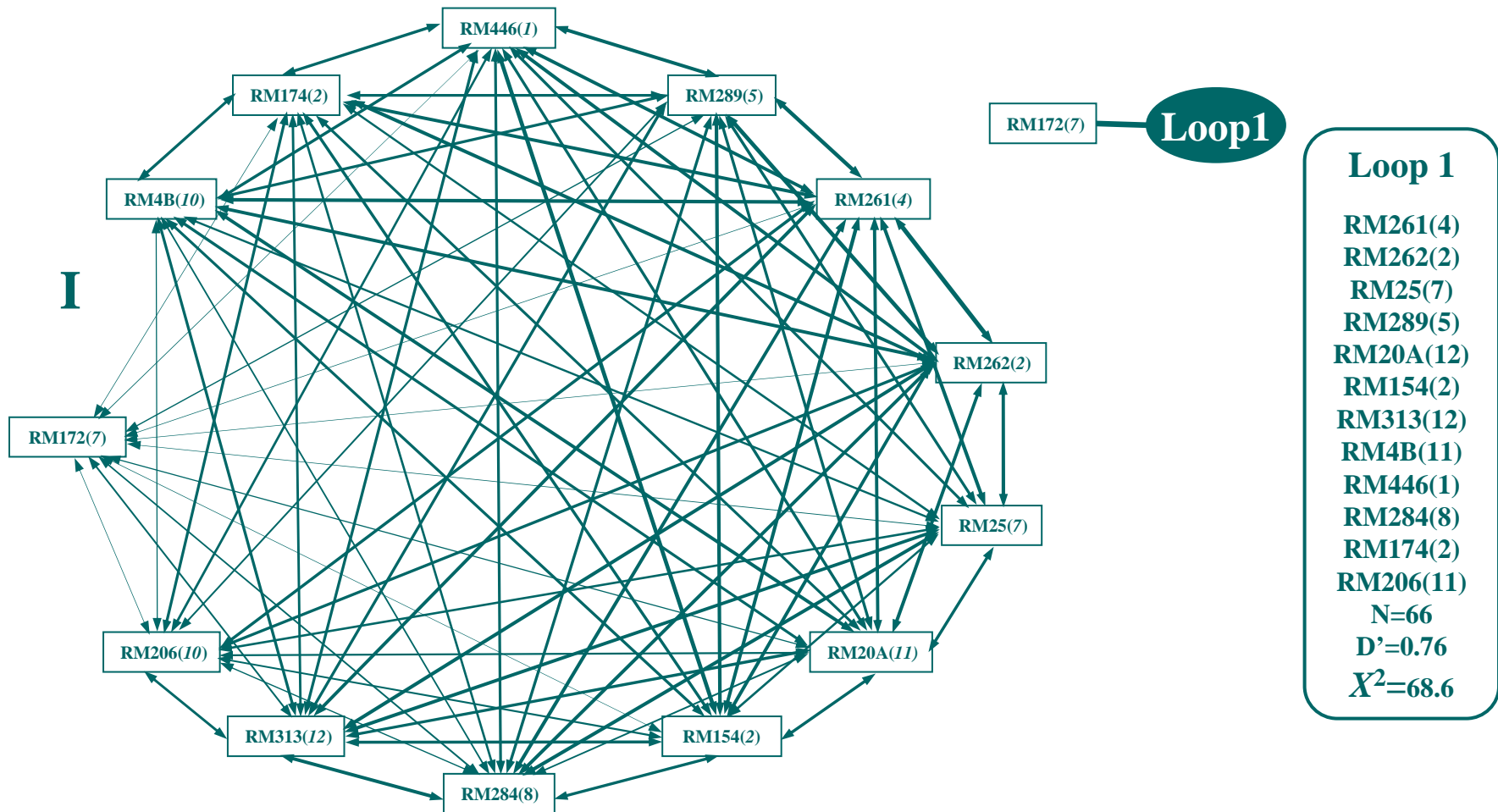
**With so many segregating DT
QTLs, how these QTLs act
individually and interactively to
contribute to DT?**

Significant LD between 33 DT QTLs in the 90 selected DT F₂ plants in population 2

P	Expected	Observed number of significant LD			
		Positive	Mean D'	Negative	Mean D'
< 0.0001	0.0095	114 (442.1)	0.67±0.32	66 (221.1)	0.69±0.23
< 0.00001	0.00095	105 (4421.1)	0.67±0.32	46 (2210.5)	0.69±0.23

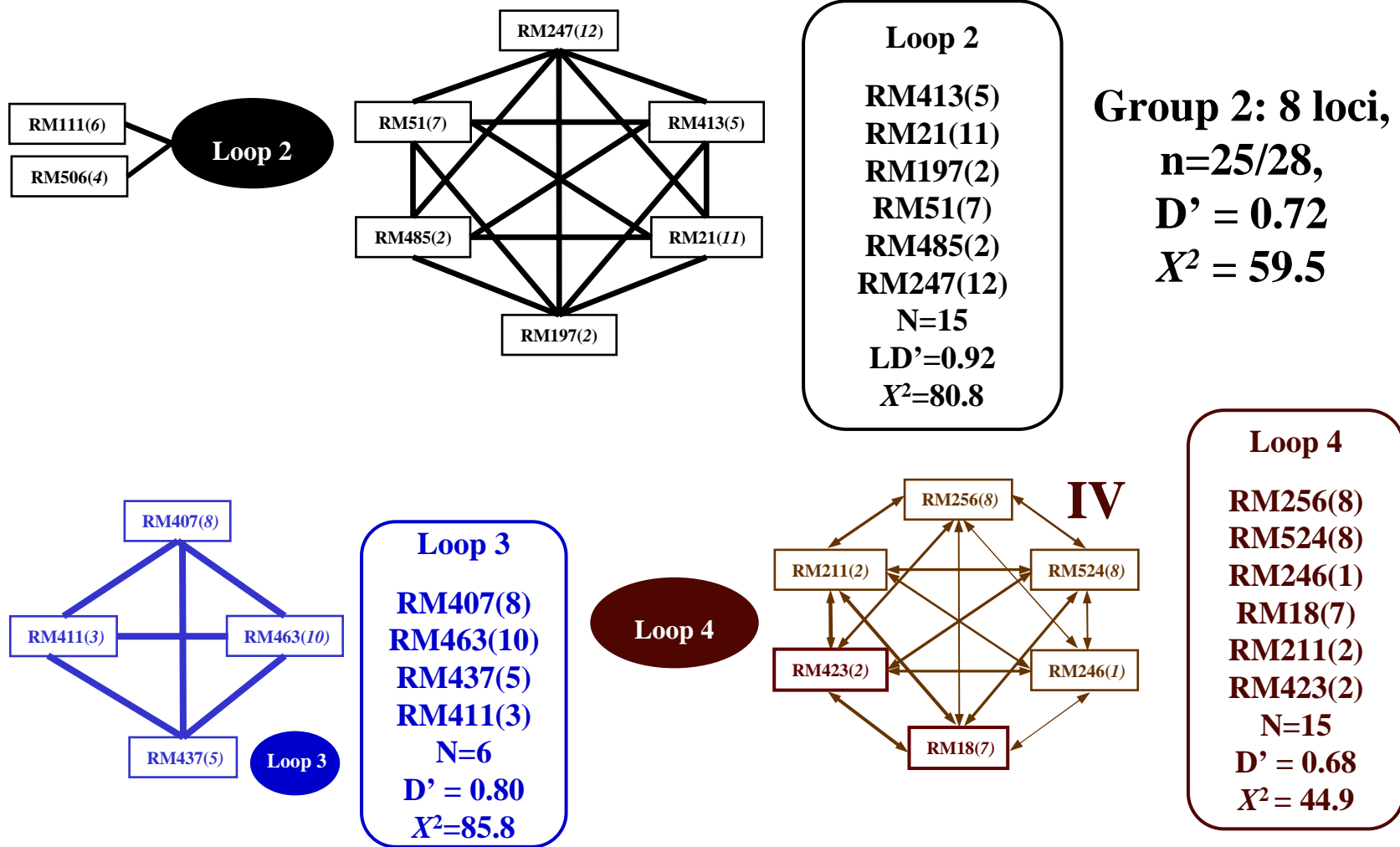
Pronounced non-random associations exist among the DT QTLs
2 (positive LD) vs 1 (negative LD)

Haplotype group I consisting of 13 largely unlinked but highly and positively associated DT loci

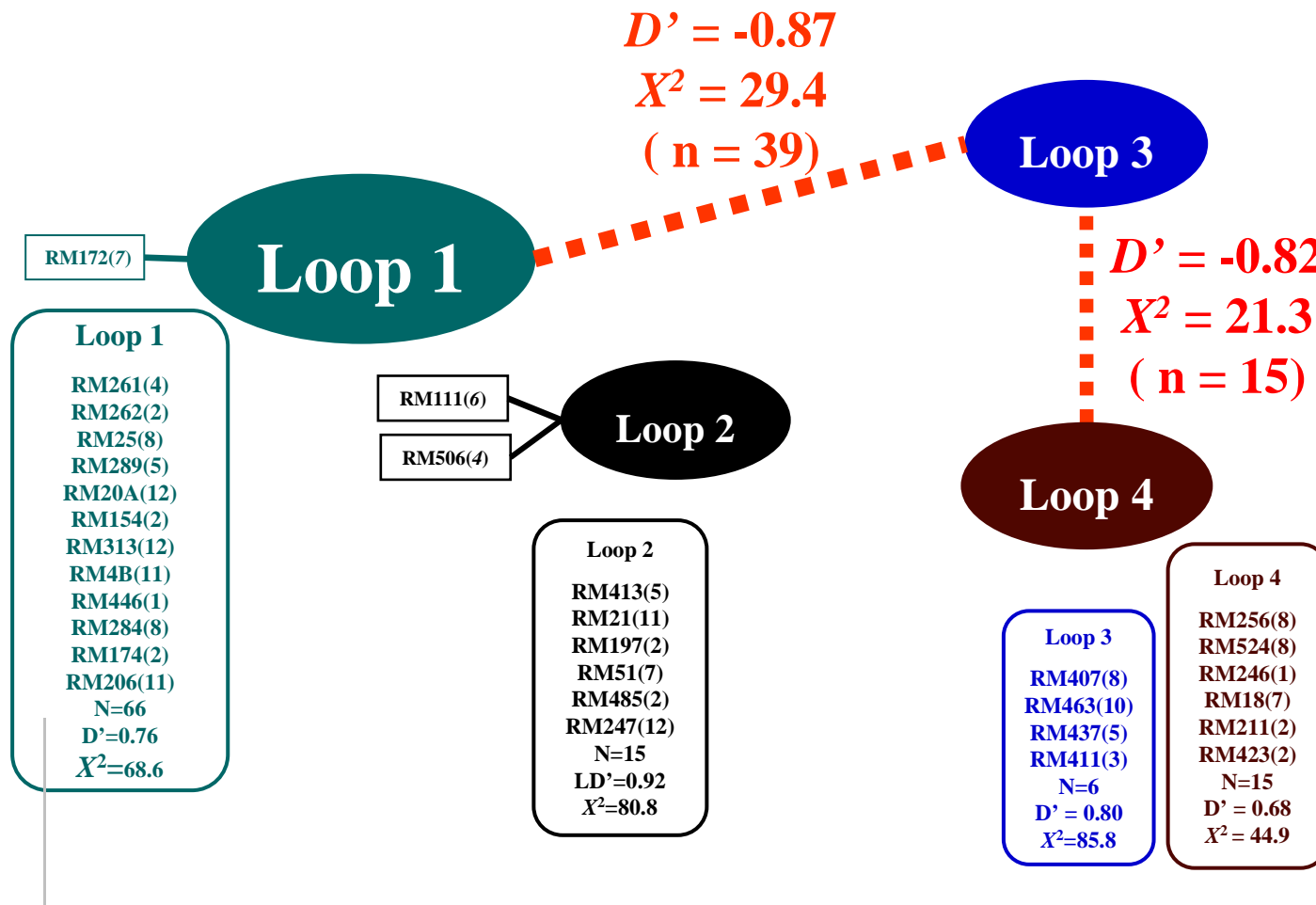


Group 1: 13 loci, N=76/78, P < 0.00001, D' = 0.72, X² = 59.5

DT haplotype groups II, III, and IV each consisted of 8, 4, and 6 unlinked but highly and positively associated loci.

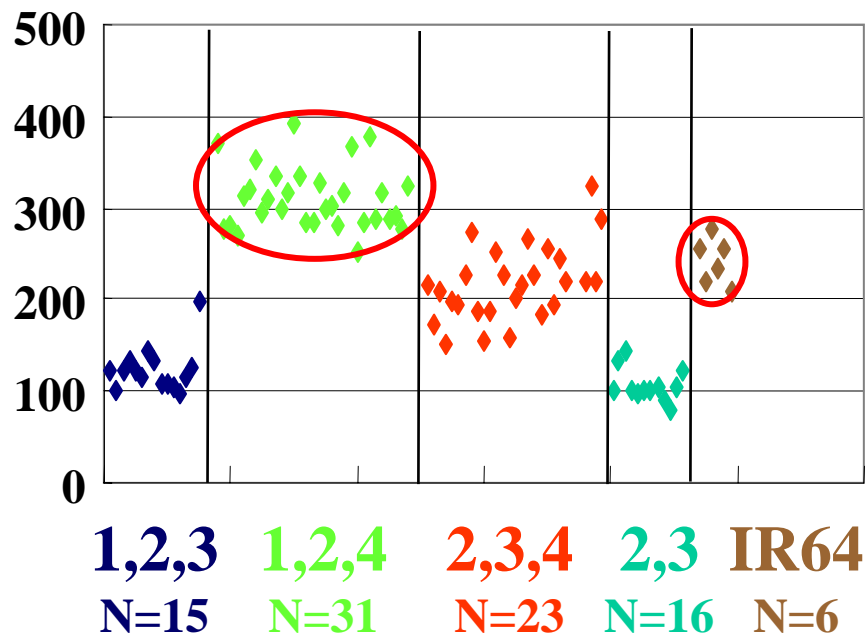


The high-confidence genetic network consisting 4 QTL groups and 31 loci contributing to drought tolerance

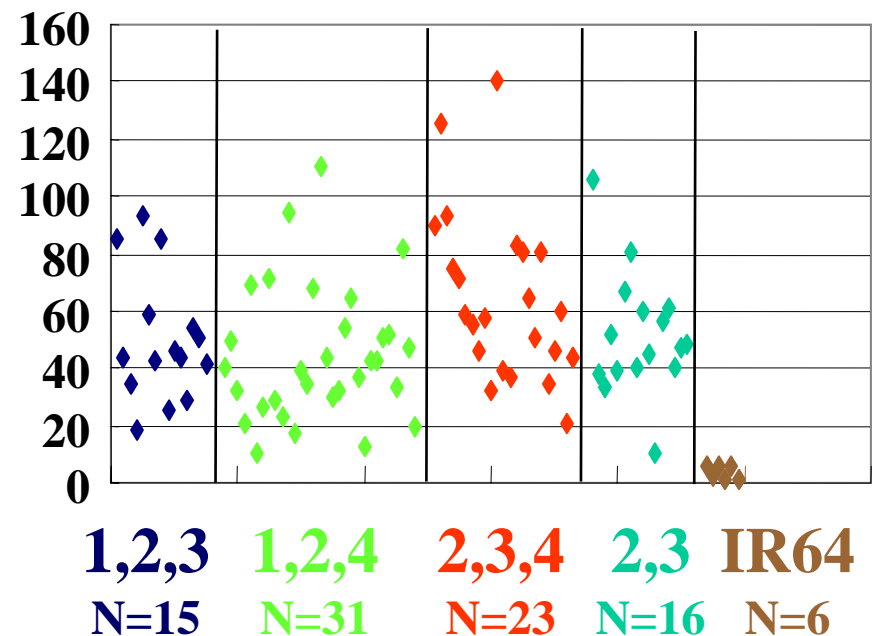


Yield performance of group genotypes under stress and non-stress conditions

Control



Stress



Summary

- 1. We have demonstrated a highly efficient strategy to put large numbers of QTLs together;**
- 2. We have identified QTL groups consisting of 32 loci (groups 1,2,4) that yield ~30% more under non-stress conditions with significantly improved DT.**
- 3. We have developed ~50 promising lines that yield significantly more than IR64 under drought and non-stress conditions.**

Conclusions

- There are tremendous amounts of hidden genetic diversity for DT in the primary gene pool of rice;
- Backcross breeding, effective selection combined with DNA markers are the effective way to discover and exploit this hidden diversity.
- Although the genetic basis of DT in rice is complex – controlled by complex genetic networks and large numbers of loci, strong selection and marker-tracking can identify the best gene combinations for simultaneous improvement of DT and yield potential.

Breeding and gene/QTL discovery can be and should be integrated.

GCP Project

**Target Area 1: ~30 M ha of the
rainfed rice of South/Southeast Asia**

Goal of Our GCP project

- **To apply BC breeding and rounds 1 and 2 strategy to developing DT japonica rice cultivars for North China;**
- **To continue round 3 to pyramid 40+ QTLs from 4 different donors in the IR64 background to develop superior DT and high yielding cultivars for the rainfed areas of South/Southeast Asia**

Participating Chinese Institutions

Activities: Parts I and II

- 1. Developing ILs : RRI of LAAS AU,
SAU, RRI of TAAS;**
- 2. Screening, Genotyping and QTL
identification and round-1 QTL
pyramiding (QTLs from two sources):
CAAS**

**The lowland nursery for screening DT ILs and
pyramiding progenies**

Progeny testing of selected DT introgression lines (ILs) and development of intercross populations:

A total of 630 ILs from 74 BC populations were progeny tested under both stress and normal irrigated conditions and 26 promising ILs were identified. A total of 46 crosses have been made between these promising ILs.

Genotyping of the selected DT lines: Genotyping of 582 DT ILs from 23 BC populations and the 25 parental lines with 600 anchor SSR markers are in progress and will be completed by before March 2006. The F_1 plants of the 46 crosses were planted into the field and each was examined with 5 polymorphic SSR markers. F_2 populations will be screened under severe stress during the dryseason of 2005-2006 to select DT lines with pyramided DT QTLs from 2 different donors.

**Seed production of hybrid rice
using a DT IL as the restorer**

**New hybrid rice cross using
DT IL as the restorer**

Research Activities and Progresses at IRRI

A total of 21 crosses were made between the 7 DT IR64 lines each with 20+ QTLs from two donors. The F1 plants of the 21 crosses were examined with at least 5 polymorphic SSR markers. F2 populations will be obtained by Nov. of 2005 and screened under very severe stress during the dryseason of 2005-2006 to select DT lines with pyramided 40+ DT QTLs from 4 different donors and for development of DT and high yielding lines for the rainfed areas of South/Southeast Asia.

A total of 30 crosses were made between a high yielding cultivar, Swarna, and DT IR64 ILs selected from 16 populations (donors) and 4 populations were advanced to BC1 generation.

Contributors

IRRI Staff

J. Domingo
R. Maghirang
C. Aquino
E. Managat
J. Mendoza

Collaborative colleagues

R. Lafitte
A. Ismail
S. Yanagihara
G. Gregorio
G. S. Khush
N. Vera Cruz
D. Mackill

CAAS Staff

B. Y. Fu
Y.M. Gao
J. L. Xu

PDFs

S. B. Yu
W. J. Xu
CHM Vijayakumar
J. Ali
Y. C. Cho
B. Y. Fu
Y.M. Gao
J. L. Xu
D. Dwivedi

Students

B. Y. Fu
D. B. Zhong
J. L. Xu
P. Bagali
M. Arif
Y. Z. Jiang
T. Q. Zheng

Acknowledgement

Funding:

The GCP of CGIAR

The Rockefeller Foundation

'948' from MA of China