



Application of Resistance Gene Analog Markers to Analyze Genetic Structure and Diversity in Rice

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Why do we use resistance gene analog (RGA) markers?

➤ What are resistance gene analogs?

Their conserved domains are homologous to known resistance genes:

NBS-LRR (nucleotide binding site-leucine rich repeat),

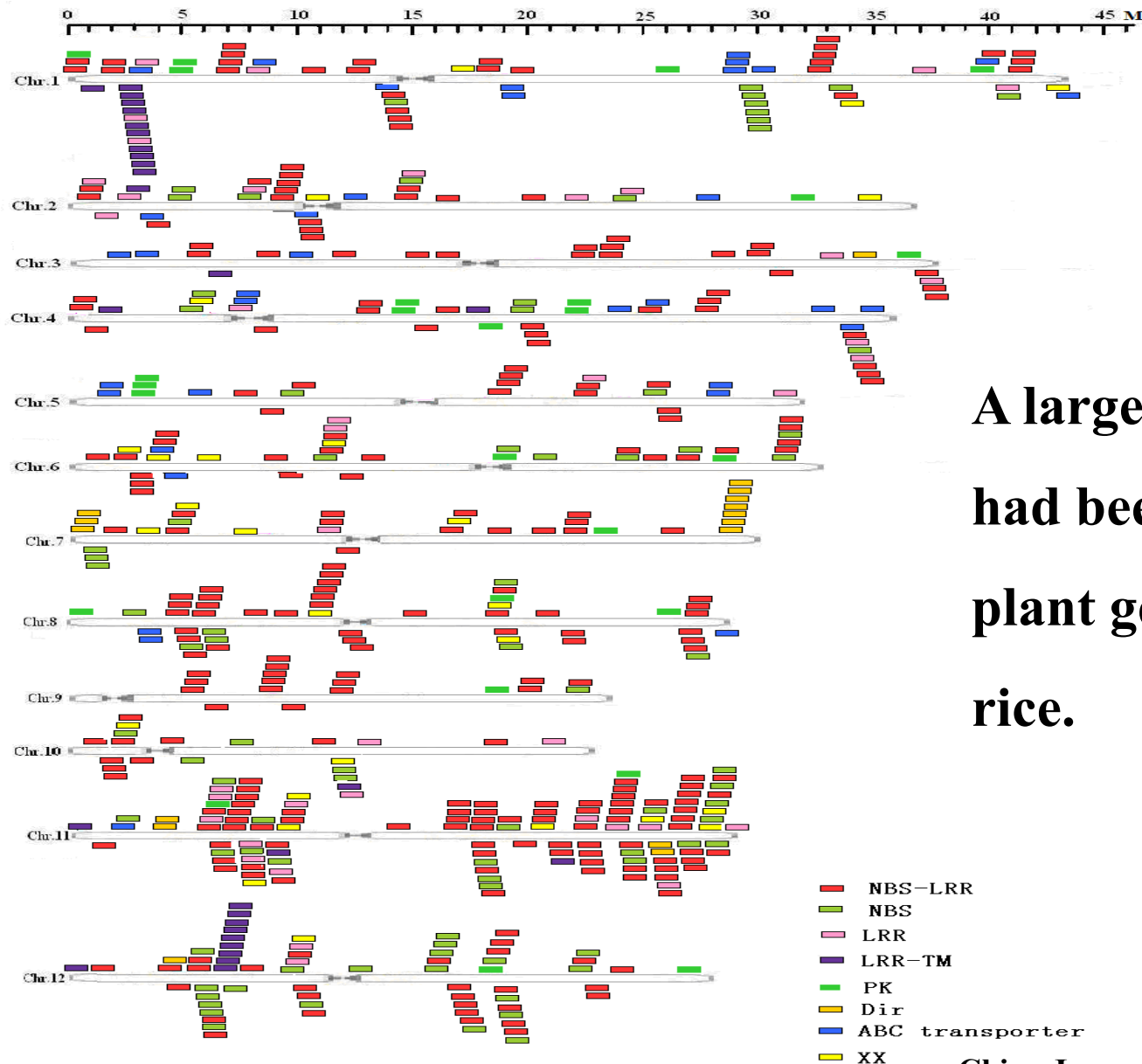
ABC (ATP-binding cassette transporter)

PK (protein kinase)

NBS TM (nucleotide binding site-transmembrane domain)

LRR TM (leucine-rich repeat-transmembrane domain)

RGAs distribution in rice chromosomes



A large number of RGAs
had been identified in
plant genome, including
rice.



Rice Genome Annotation Project

Funded by the NSF



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Search Examples:

- kinase serine - will match genes annotated with both serine kinase, with just serine or with just kinase

Putative Function Search Tool

Search Genes:

There are 404 genes matched with disease resistance

Chr	Locus Identifier	Putative Function
Chr1	LOC_Os01g02810	resistance-related receptor-like kinase, putative, expressed
Chr1	LOC_Os01g02840	resistance-related receptor-like kinase, putative, expressed
Chr1	LOC_Os01g04070	verticillium wilt disease resistance protein, putative, expressed
Chr1	LOC_Os01g05600	NBS-LRR disease resistance protein, putative, expressed
Chr1	LOC_Os01g05620	NBS-LRR disease resistance protein, putative, expressed
Chr1	LOC_Os01g06520	verticillium wilt disease resistance protein, putative, expressed
Chr1	LOC_Os01g06720	disease resistance protein SIVe2 precursor, putative, expressed
Chr1	LOC_Os01g06730	verticillium wilt disease resistance protein, putative, expressed
Chr1	LOC_Os01g06835	disease resistance protein SIVe2 precursor, putative, expressed
Chr1	LOC_Os01g06870	resistance protein SIVe1 precursor, putative, expressed
Chr1	LOC_Os01g06900	verticillium wilt disease resistance protein Ve2, putative, expressed
Chr1	LOC_Os01g06920	resistance protein SIVe1 precursor, putative, expressed
Chr1	LOC_Os01g07090	5-azacytidine resistance protein azr1, putative, expressed
Chr1	LOC_Os01g07280	disease resistance protein, putative, expressed
Chr1	LOC_Os01g08180	BRASSINAZOLE-RESISTANT 2, putative, expressed
Chr1	LOC_Os01g08260	pleiotropic drug resistance protein 4, putative, expressed
Chr1	LOC_Os01g08370	symbiosin-related disease resistance protein, putative, expressed
Chr1	LOC_Os01g16320	NBS-LRR type disease resistance protein, putative, expressed
Chr1	LOC_Os01g23380	stripe rust resistance protein Yr10, putative, expressed
Chr1	LOC_Os01g24010	pleiotropic drug resistance protein, putative, expressed
Chr1	LOC_Os01g25386	multidrug resistance-associated protein, putative, expressed
Chr1	LOC_Os01g25720	disease resistance protein RGA4, putative, expressed
Chr1	LOC_Os01g25740	powdery mildew resistance protein PM3F, putative, expressed
Chr1	LOC_Os01g25760	powdery mildew resistance protein PM3F, putative, expressed
Chr1	LOC_Os01g25810	powdery mildew resistance protein PM3A, putative, expressed
Chr1	LOC_Os01g32870	unlike resistance protein R1, putative, expressed
Chr1	LOC_Os01g36640	disease resistance protein RPM1, putative, expressed
Chr1	LOC_Os01g42350	pleiotropic drug resistance protein, putative, expressed
Chr1	LOC_Os01g42370	pleiotropic drug resistance protein, putative, expressed
Chr1	LOC_Os01g42380	pleiotropic drug resistance protein, putative, expressed
Chr1	LOC_Os01g42410	pleiotropic drug resistance protein, putative, expressed
Chr1	LOC_Os01g50920	multidrug resistance protein 1, putative, expressed
Chr1	LOC_Os01g57220	disease resistance RPP13-like protein 1, putative, expressed
Chr1	LOC_Os01g57820	disease resistance protein RPS2, putative, expressed
Chr1	LOC_Os01g65800	powdery mildew resistant protein 5, putative, expressed
Chr1	LOC_Os01g67580	multidrug resistance-associated protein, putative, expressed
Chr1	LOC_Os01g71106	NBS-LRR disease resistance protein, putative, expressed
Chr1	LOC_Os01g71114	disease resistance protein RGA4, putative, expressed
Chr1	LOC_Os01g72390	NBS type disease resistance protein, putative, expressed
Chr1	LOC_Os01g72680	disease resistance protein RPS2, putative, expressed
Chr2	LOC_Os02g02640	NBS-LRR disease resistance protein, putative, expressed
Chr2	LOC_Os02g02670	NBS-LRR disease resistance protein, putative, expressed
Chr2	LOC_Os02g04530	unlike resistance-like protein R1, putative, expressed
Chr2	LOC_Os02g11760	pleiotropic drug resistance protein, putative, expressed
Chr2	LOC_Os02g16070	lri disease resistance protein, putative, expressed
Chr2	LOC_Os02g16250	NBS-LRR disease resistance protein, putative, expressed
Chr2	LOC_Os02g17304	resistance protein, putative, expressed
Chr2	LOC_Os02g18000	disease resistance protein RGA2, putative, expressed
Chr2	LOC_Os02g18140	NBS type disease resistance protein, putative, expressed
Chr2	LOC_Os02g18510	stripe rust resistance protein Yr10, putative, expressed
Chr2	LOC_Os02g19250	NBS-LRR type disease resistance protein, putative, expressed
Chr2	LOC_Os02g21350	pleiotropic drug resistance protein 5, putative, expressed
Chr2	LOC_Os02g27710	disease resistance protein, putative, expressed

The identified RGAs were marked for gene expression and coding resistance protein in TIGR rice.

RGA markers are associated with disease resistance

- **RGA marker primers can be designed based on the conserved sequences of known resistance genes or the exon sequences of **resistance gene analogs**.**

Rice is seriously suffered from many pathogens and insects



Blast



Sheath blight



False smut



Bacterial blight



Dwarf virus



Brown planthopper

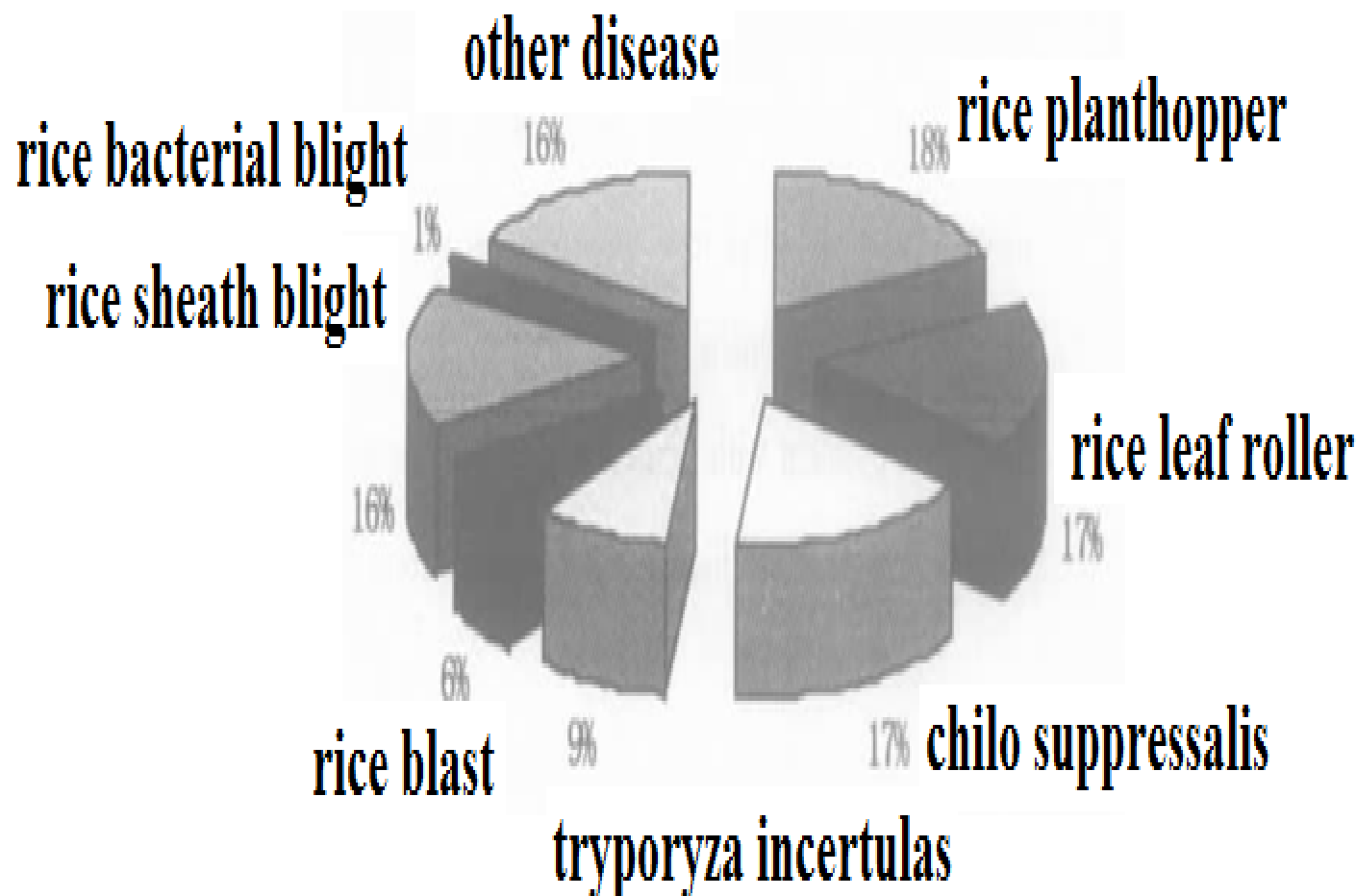
Diseases and insects cause rice yield losses



In **China**, 4~5 million tons of the losses each year.

Diseases

Insects



➤ For the first time, 472 genome-wide RGA markers were used to analyze 178 USDA rice core entries for genetic variations related to their ancestral backgrounds and geographic distributions.

➤ Comparison of the results from RGA markers with SSR markers will confirm if RGAs could be used as a new application.

Materials and methods



They were selected for genotyping because they are well adapted in Sichuan area with many interesting traits.

178 USDA rice core entries were planted in Chengdu

See USDA rice core collection in 2007 *Crop Science* 47: 869-878



Methods

➤ Design RGA markers

472 genome wide RGA markers were designed by Primer Premier 6.0.

➤ Genotyping

178 core entries were genotyped with the 472 RGA markers.

➤ Structure and diversity

The genotypic information is used to analyze population structure and genetic diversity among 178 core entries.



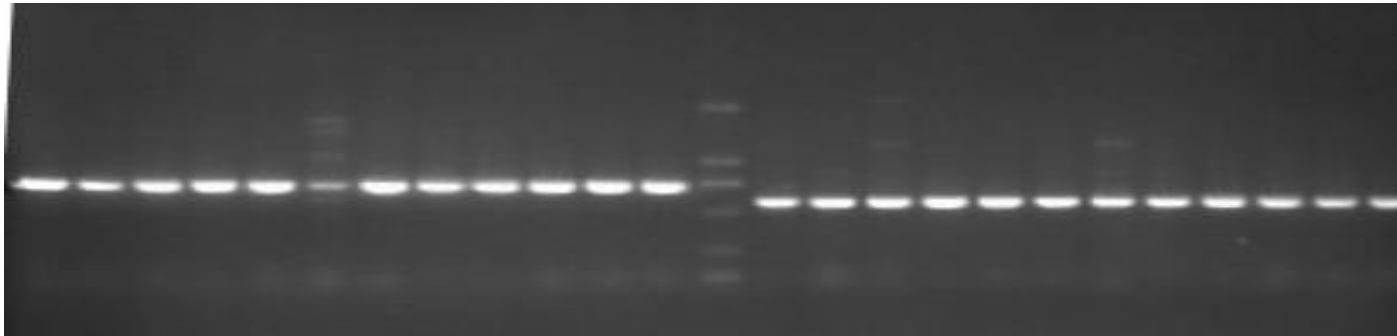
Results

RGA marker polymorphism

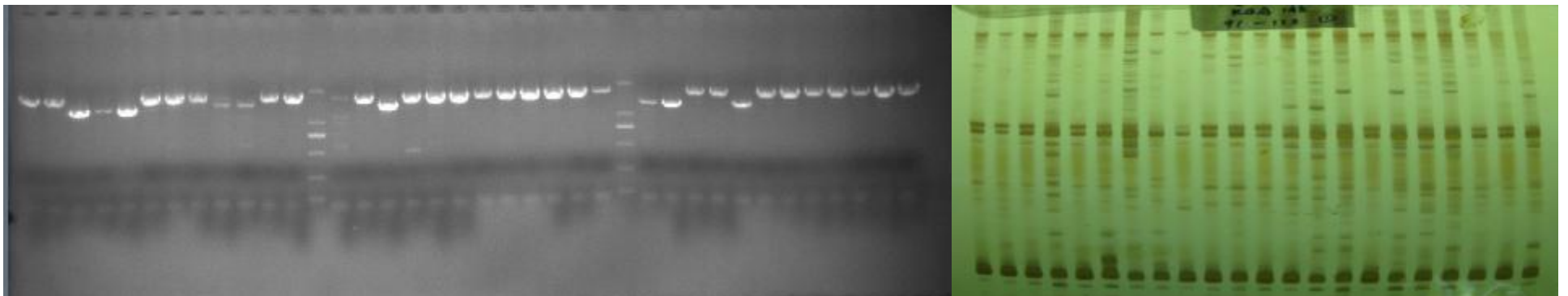
Among 472 RGA markers

- 21 failed to be amplified**
- 451 yielded 719 DNA bands**

➤ Among 451 RGAs, 313 were monomorphic

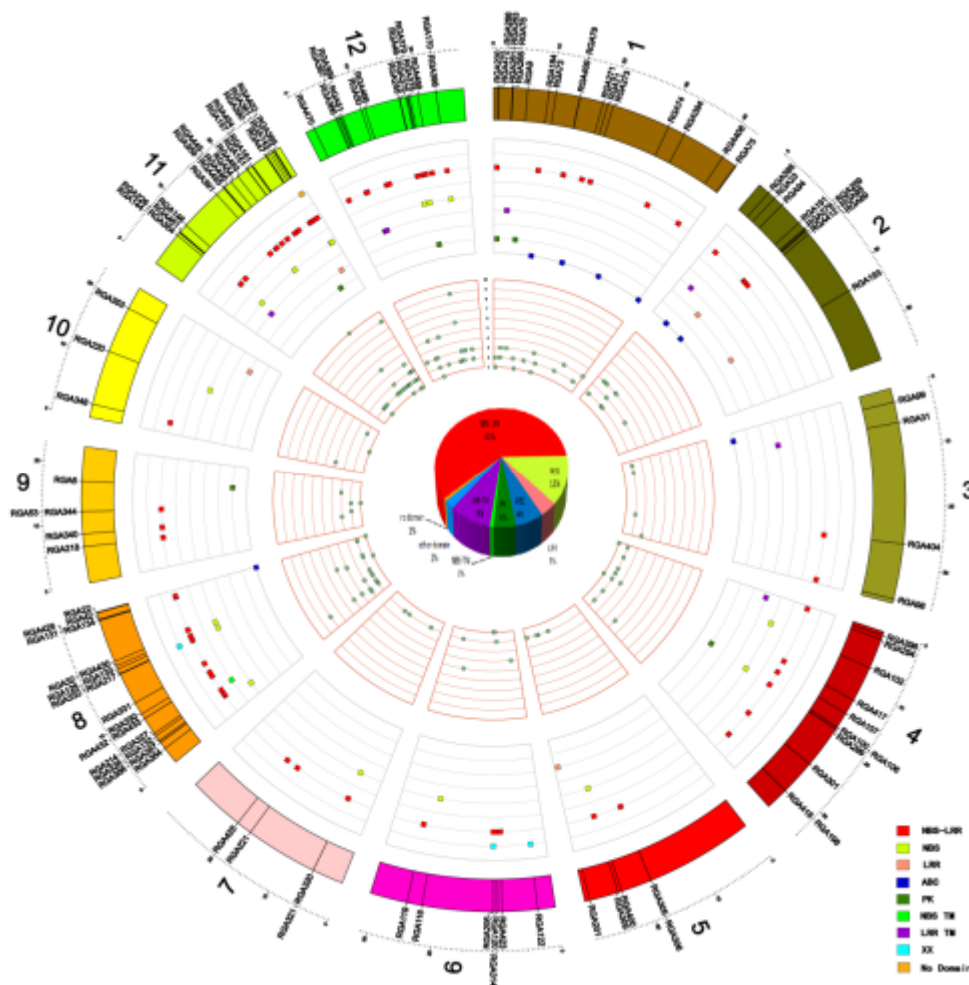


➤ 138 RGAs were polymorphic. They yielded 372 alleles with 96% of polymorphic alleles



Genome distribution of polymorphic RGA markers

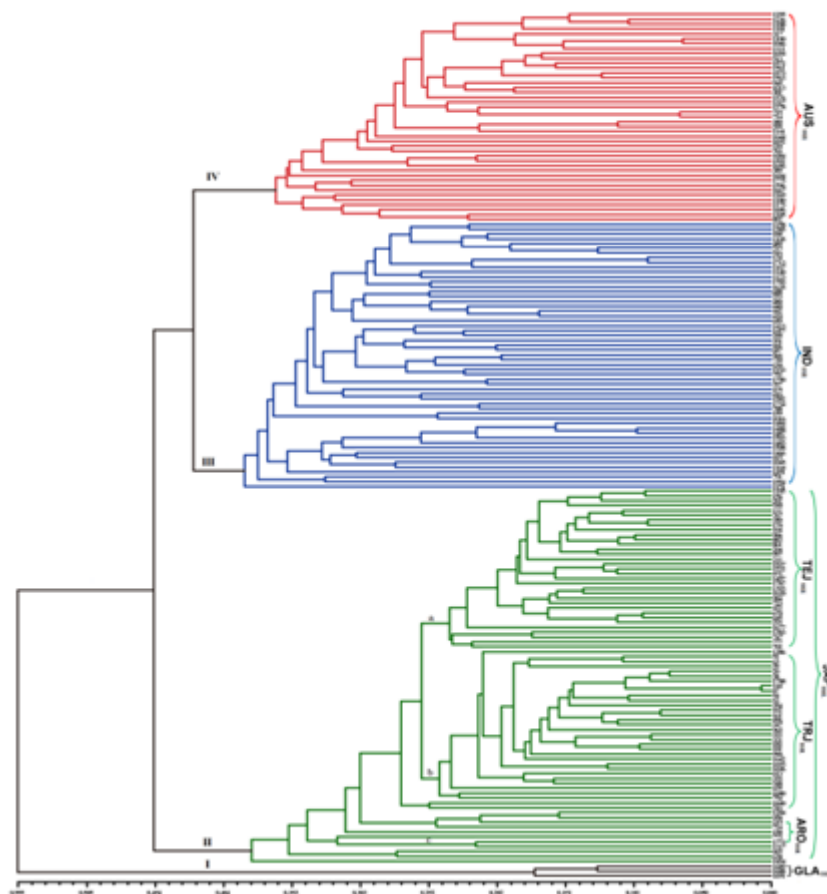
- ✓ Inner ring for polymorphism
- ✓ Outer ring for protein domain
- ✓ Pie chart for percentage of polymorphic RGA markers



■	NBS-LRR	60%
■	NBS	13%
■	LRR	9%
■	ABC	6%
■	PK	5%
■	NBS TM	4%
■	LRR TM	2%
■	XX	1%
■	No Domain	1%

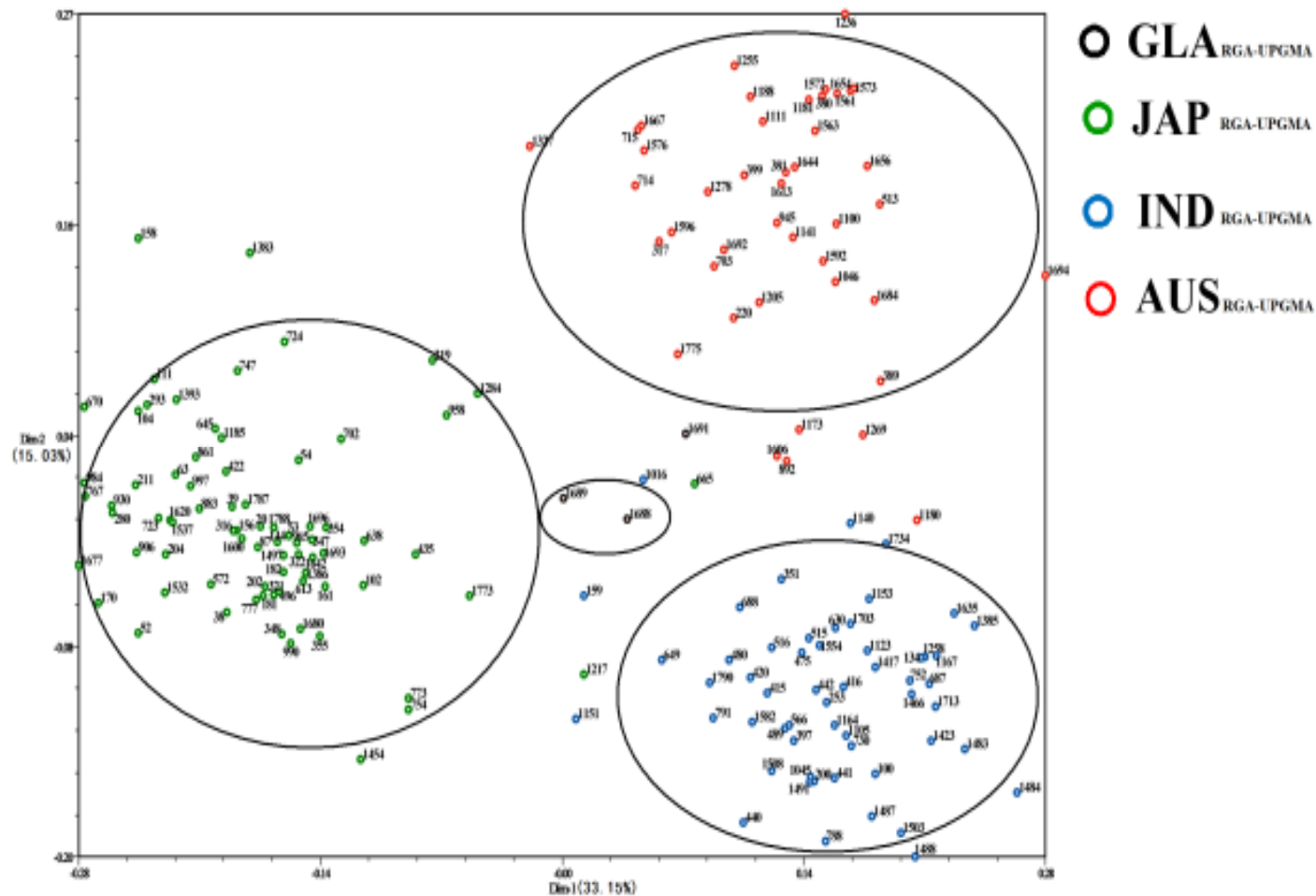
Population structure of 178 core entries

138 RGA markers classified 178 core entries into four subgroups using four methods.



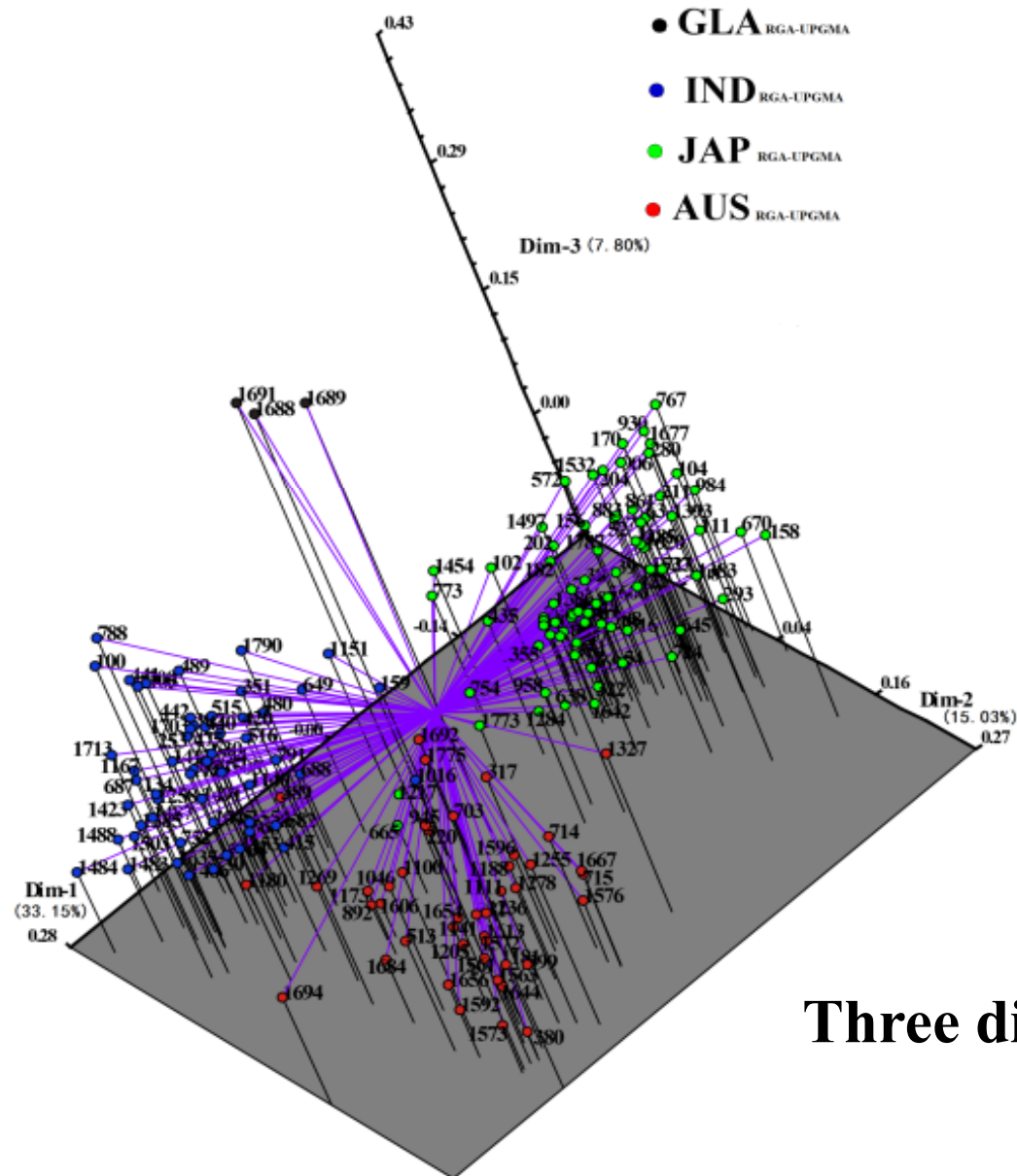
UPGMA

Population structure of 178 core entries

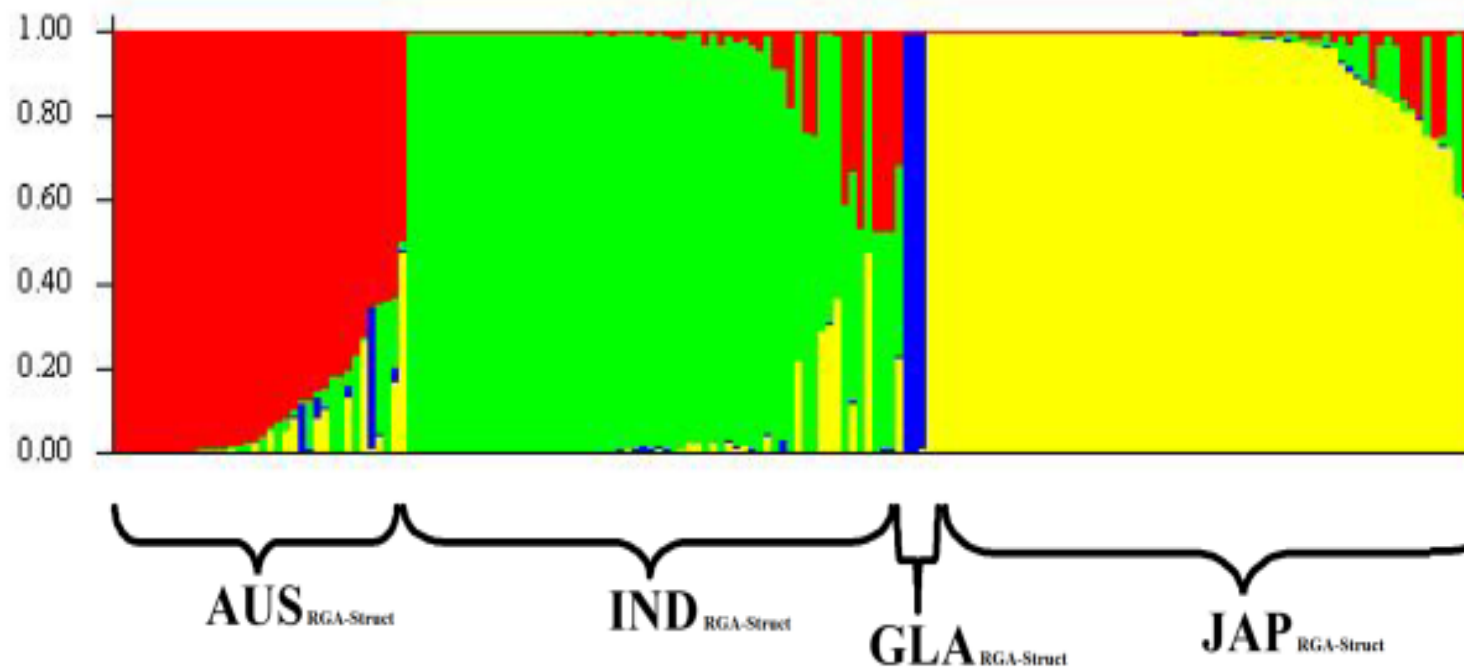


Two dimensional PCA

Population structure of 178 core entries



Population structure of 178 core entries



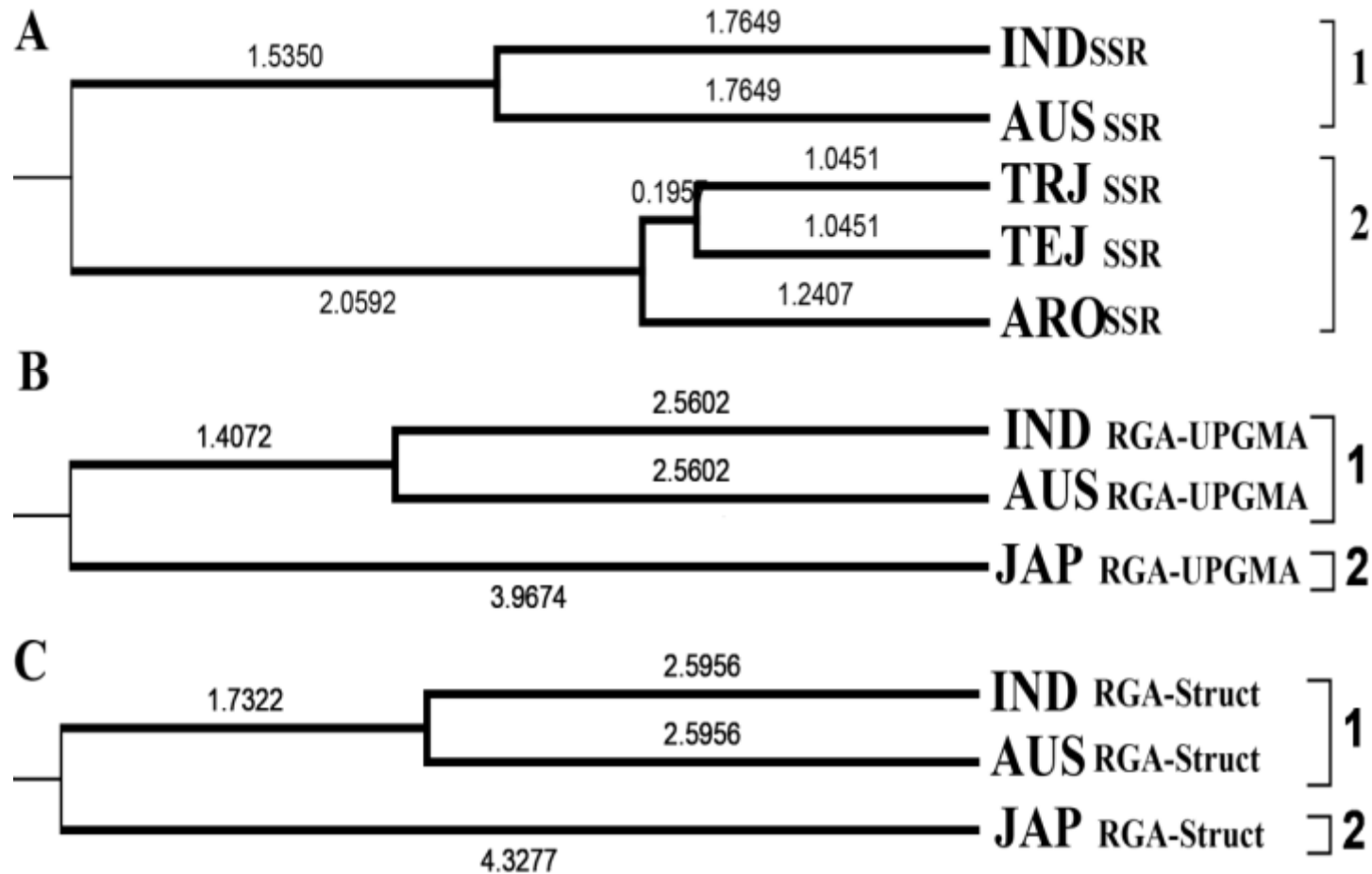
STRUCTURE

Comparison of subgroups by RGA markers and SSR among 178 US rice core entries

Subgroup	IND _{SSR}	TEJ _{SSR}	TRJ _{SSR}	AUS _{SSR}	ARO _{SSR}	Total
AUS _{RGA-UPGMA}	7			35	1	43
IND _{RGA-UPGMA}	47	2	3	1		53
JAP _{RGA-UPGMA}	5	32	31	3	8	79
GLA _{RGA-UPGMA}				3(GLA)		3
AUS _{RGA-Struct}	1			36	1	38
IND _{RGA-Struct}	56	4	3	1		64
JAP _{RGA-Struct}	2	30	31	2	8	73
GLA _{RGA-Struct}				3(GLA)		3
Total	59	34	34	42	9	178

SSR results from 2010 Natural Science 2(4):247-291

Comparatively cluster analysis between SSRs (A) and RGAs (B and C)

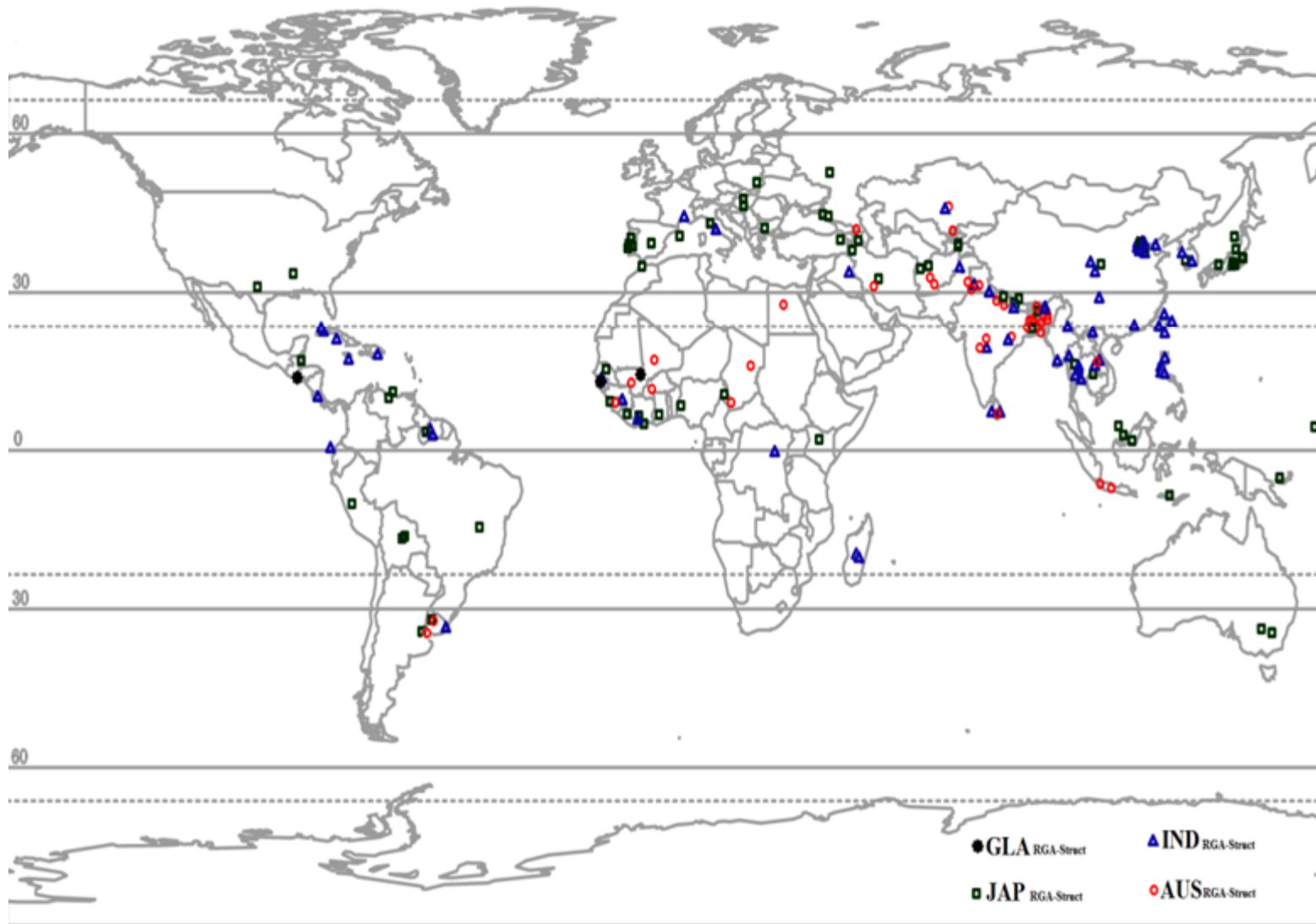


Groups of A are from 2010 Natural Science 2(4):247-291

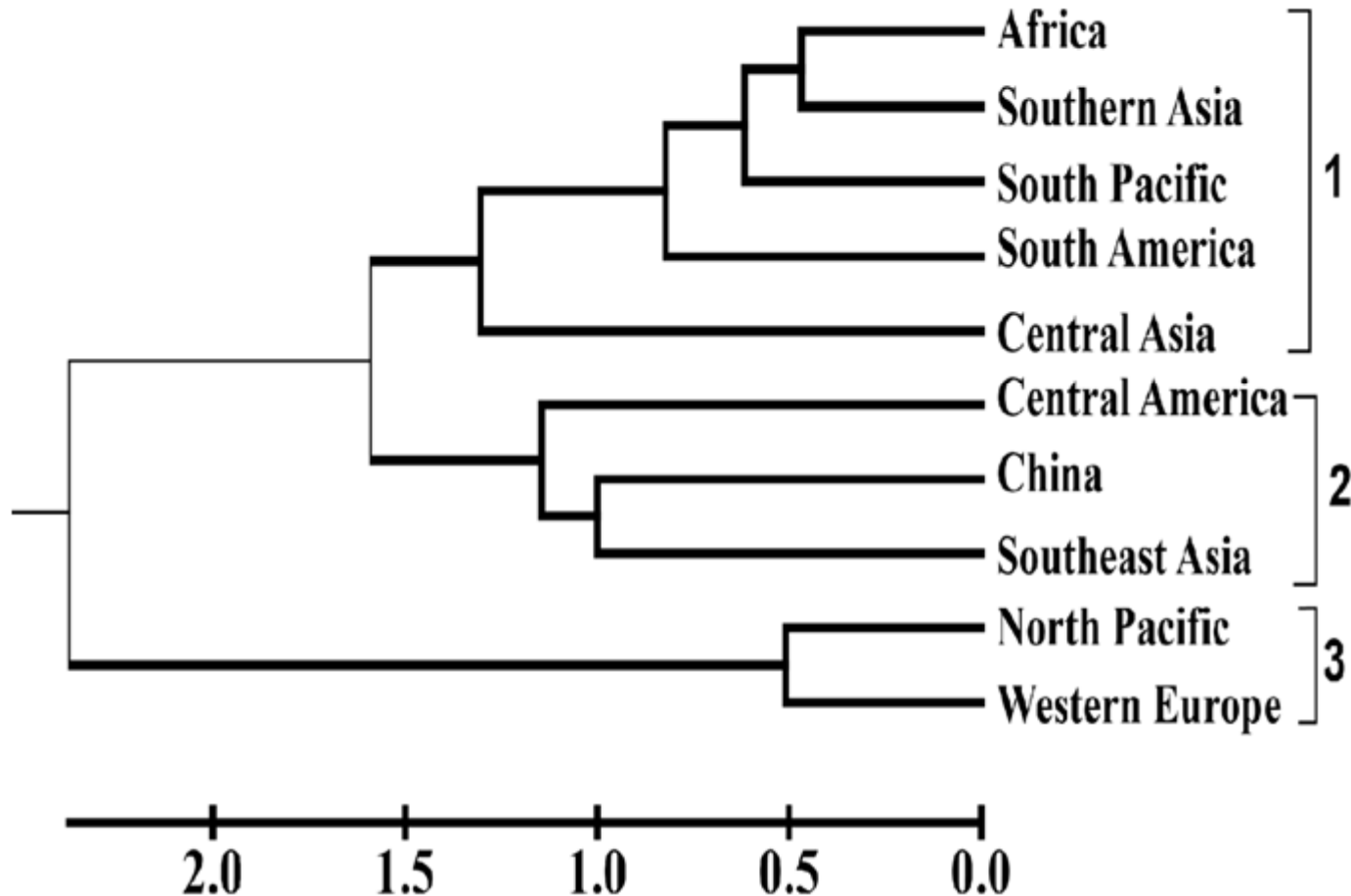
Comparative analysis of genetic variations among subgroups classified by SSR markers and RGA markers

Subgroup	Entries	PPA%	Na	Ne	H	I	Nm*
IND _{SSR}	59	82.80	1.8280	1.3703	0.2247	0.3476	
TRJ _{SSR}	34	69.84	1.6984	1.3056	0.1848	0.2866	
TEJ _{SSR}	34	72.49	1.7249	1.3253	0.1952	0.3009	2.9210
AUS _{SSR}	42	84.13	1.8413	1.3773	0.2254	0.3477	
ARO _{SSR}	9	53.70	1.5370	1.2864	0.1712	0.2608	
Mean			1.9603	1.4032	0.2429	0.3761	
St. Dev			0.1955	0.3527	0.1796	0.2409	
IND _{RGA-UPGMA}	53	79.10	1.7910	1.3497	0.2122	0.3284	
JAP _{RGA-UPGMA}	79	80.16	1.8016	1.3236	0.1977	0.3077	2.7896
AUS _{RGA-UPGMA}	43	78.04	1.7804	1.3461	0.2085	0.3225	
GLA _{RGA-UPGMA}	3	16.93	1.1693	1.1100	0.0642	0.0953	
Mean			1.9603	1.4065	0.2442	0.3777	
St. Dev			0.1955	0.3553	0.1803	0.2414	
IND _{RGA-Struct}	64	83.07	1.8307	1.3643	0.2212	0.3425	
JAP _{RGA-Struct}	73	75.93	1.7593	1.3042	0.1864	0.2902	2.5546
AUS _{RGA-Struct}	38	73.02	1.7302	1.3350	0.2002	0.3081	
GLA _{RGA-Struct}	3	16.93	1.1693	1.1100	0.0642	0.0953	
Mean			1.9603	1.4050	0.2438	0.3773	
St. Dev			0.1955	0.3534	0.1796	0.2407	
Total	178	96.03					

Global distributions of four subgroups from RGA markers using STRUCTURE



Cluster analysis of ten geographic regions using RGA markers





Summary

- RGA markers can be used for analyses of genetic structure**
- RGA markers have less discriminatory power than SSR markers**
- RGA markers can reveal molecular basis of rice subgroup differentiation in pathogen resistance**

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**Thank you for your
attention!**

Hybrid rice