PROJECT NO. RB-3

ANNUAL REPORT
COMPREHENSIVE RESEARCH ON RICE
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PROJECT TITLE: Rice Genetics and Germplasm Development

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OBJECTIVES AND EXPERIMENTS CONDUCTED BY LOCATION TO ACCOMPLISH OBJECTIVES:

1. **Rice Genetic Resources**. Objectives are to maintain and evaluate a diverse set of rice varieties and wild species, import useful new germplasm and introduce useful traits into California varieties.

   1993 Experiments:

   1. Field-testing a diverse set of rice varieties from the germplasm collection.

   2. Completing a molecular survey of temperate rice germplasm.

   3. Making crosses between japonica cultivars and exotic varieties/species to introduce useful genes.

2. **Identification of useful genes.** The main strategy we use for identifying useful genes is to use DNA markers to "tag" important genes, as described above for the germplasm survey.

   1993 Experiments:

   1. Genetics of resistance to stem rot.

   2. Genetics of cold tolerance.

   3. Genetics of seedling vigor.

3. **Hybrid rice.** Genetic mechanisms have now been developed for commercial hybrid rice production. Whether these will be viable in California depends on the cost of seed production and the availability of a hybrid with sufficient yield advantage and acceptable grain quality. Our research focuses on genetic mechanisms of seed production.

   1993 Experiments:

   1. Transfer of cytoplasmic male sterility (cms) and restorer genes into California adapted germplasm.

   2. Development of photoperiod-sensitive genic male sterility (PGMS) for hybrid rice seed production.

   3. Transfer of wide compatibility genes into California cultivars.

SUMMARY OF 1993 RESEARCH (MAJOR ACCOMPLISHMENTS) BY OBJECTIVE

1. **Rice genetic resources**

   Rices from diverse sources were evaluated in the field at Davis (Table 1). Data was collected on agronomic characters. Useful varieties were selected for crossing or further evaluation (Table 2). Crosses with exotic parents have also been made to develop useful germplasm or for breeding studies (Table 3).

   A type of molecular marker termed RAPD (random amplified polymorphic DNA) was used to classify a set of 138 rice cultivars. Cluster analysis showed general agreement with the currently used indica-japonica classification, with javanica types being a subclass of japonica (Fig. 1, Table 4). Three subgroups were identified within japonica: (1) japonica 1 or "javanica", consisting of tropical japonicas and US long grain varieties, (2) japonica 2, a smaller group consisting mostly of California medium grain types and (3) japonica 3 which contains most of the traditional and improved temperate japonica varieties. US cultivars were classified in more detail with an additional set of markers. Almost all varieties could be distinguished from each other (Fig. 2). The conclusions were: (1) RAPDs are suitable for classifying varieties into major groups, but many japonica varieties (including California medium grains) could not be differentiated from each other. RAPDs are not an ideal method for differentiating closely related japonica varieties. (2) Since heterosis seems to be related to genetic diversity, parents from different groups may represent potential parents for hybrid rice varieties. The most likely source of hybrid vigor would be medium (or short) crossed with long grain varieties. (3)

RAPDs will be excellent markers for tagging genes in wide crosses (i.e. indica X japonica or crosses with wild species) but will be difficult to use within conventional japonica varieties. A different type of marker should be sought for these types of studies.

2. **Identification of useful genes**

Stem rot resistance. Populations which possess resistance from 87Y550 (from *Oryza rufipogon*) are being advanced at Biggs. Within one year these should be ready for evaluation for stem rot resistance. RAPD analysis shows that 87Y550 is very close genetically to California long grain varieties L202 and L203 (Fig. 2). It seems that *O. rufipogon* is genetically distant from California japonicas (Fig. 1). This implies that O. rufipogon segments conferring resistance should be tagged easily with molecular markers. A more detailed survey with RFLPs and RAPDs will be conducted on these parents.

Cold tolerance. The initial strategy was to develop a doubled haploid population through anther culture, but the response of the F_1 populations was very poor, so a sufficient number of plants could not be regenerated. An F2 population will be grown in the winter of 1994 and evaluated for cold tolerance in the growth chamber. This should form the basis for tagging cold tolerance genes.

Seedling vigor. RFLP analysis is now being conducted on an F_2 population which carries a tropical source of seedling vigor. Initial results from this study will be available within a few months. The F_3 progeny will be evaluated in 1994.

3. **Hybrid rice**

The basis of the so-called 3-line method of seed production involves cytoplasmic male sterility (cms). In order to utilize this method we need to transfer cms and restorer (R) genes into the appropriate parents. We are currently transferring two types of cms into California varieties (Table 5). Our studies confirm that California medium grains are maintainers, as the F_1 plants are always sterile. R genes are not present in japonica cultivars so we are transferring them from tropical sources (Table 5).

The 2-line method of hybrid seed production requires an environmentally-sensitive form of genetic male sterility. This is usually termed PGMS (for photoperiod-sensitive genic male sterility), although research has shown that both daylength and temperature play a role in sterility induction. Sterility is induced by long days and/or high temperature. Two PGMS mutants have been developed at Davis. Our research indicates that neither would be suitable for hybrid seed production in California (Table 6). We are currently crossing these mutants into other genetic backgrounds to see if more useful PGMS types can be developed. We are also attempting to tag the PGMS genes with molecular markers.

Wide compatibility (WC) is a useful trait for hybrid rice and also for using indica X japonica crosses. We are currently introducing this gene from the variety Lemont into California medium grains.

PUBLICATIONS OR REPORTS:

Mackill, D. J., Rutger, J. N. and Johnson, C. W. 1992. Elongated uppermost internode (*eui*) is a recurring mutation. Rice Genet. Newsl. 9:102-103.

Mackill, D. J., Pinson, S. R. M. and Rutger, J. N. 1992. Frizzy panicle, an EMS-induced mutant in the japonica cultivar M-201. Rice Genet. Newsl. 9:100-102.

Mackill, D. J. Classification of rice cultivars by RAPD markers. 1993. p. 114. *In* Seventeenth International Congress of Genetics, Volume of Abstracts. 15-21 Aug 1993. Birmingham, United Kingdom.

Mackill, D. J., and Lei, X. 1993. Research on hybrid rice in California. p. 16. *In* Rice Field Day, 2 Sep 1993, Rice Experiment Station, Biggs, CA.

Mackill, D. J. 1993. Characterization of rice cultivars with molecular markers. p. 191. Agronomy Abstracts 1993:191.

CONCISE GENERAL SUMMARY OF CURRENT YEAR'S RESULTS:

Research on plant genetic resources must form the base of any long-term breeding effort. We spent considerable time in maintaining and evaluating potentially useful varieties and we expect this work to continue in the future. In 1993, useful information was obtained from the molecular survey of US rice germplasm. The potential for identification of US rices with DNA markers was demonstrated. These varieties were classified into groups based on genetic background, and this information will be used in future studies on gene mapping and hybrid vigor.

The foundation for tagging useful genes with molecular markers was laid. We are moving rapidly toward identifying genes for stem rot resistance and seedling vigor, and we expect that cold tolerance will not be far behind. We are also preparing populations for water weevil tolerance and other useful traits. We are beginning work on using new types of markers for tagging useful genes. The goal is to have an easy means of selecting for genes that are very difficult to detect.

Hybrid rice production in California is a long term goal. While considerable bottlenecks exist the potential yield increase from heterosis warrants continued research. Progress in other locations indicates that hybrid varieties may soon be a reality outside of China. We have begun to develop the components that will allow us to assess the suitability of the technology for increasing rice yields in California. These include transferring of cytoplasmic male sterility (cms), restorer gene and wide compatibility into California varieties and evaluation and further development of photoperiod-sensitive genic male sterile lines. In 1994, cms lines in California background will be available and their application in hybrid seed production will be studied. Crosses for transferring the restorer gene have been made but further backcrosses to appropriate cultivars are needed.

Table 1. Germplasm evaluated in field trials at Davis, 1993. Data were collected on seeding vigor, plant height, days to flowering and blanking.

Description	Origin	Source	Number
1. Traditional/improved var.	Korea	H.P. Moon	36
2. Japonica germplasm	China Korea Japan Europe	Nat. Small Grain	222
3. Core collection	Various	IRRI, Philippines	244
4. Survey experiment	Various	Various	119

Table 2. Some of the promising (or "interesting") varieties identified in field screening at Davis, 1993.

Description	Acc. no.	Origin	Days to heading	Comments
Akceltik	172526	Turkey	95	Large grains
Baldo	388247	Brazil	95	Good stems
Batakta	189458	Portugal	104	Strong stems
Carina	350296	Bulgaria	98	
Cume Man Fa	164910	Argentina	103	
Gogo Lempuk	F1211*	Indonesia	111	Early javanica
Hunan Early Dwarf 7		China	87	Good indica type
Hunan Sinica 12		China	99	Good indica type
Jhona 349	F1288*	India	108	High blanking (susc. ck.)
Kotobuki mochi	F998*	Japan	118	Large grains
Murasaki Ine	224844	Japan	110	Purple
Qing Gan Huang	503038	China	104	Geese like it
Quing Qun Wang		China	103	Good indica type
Uspeh	350302	Bulgaria	91	

* imported direct from IRRI in 1991

Table 3. Crosses made with exotic parents for introgressing new genes into California japonicas.

Cross	CA parent	Exotic parent	Comments
DX9	C76	Jalamagna	Deepwater variety, escape from deep flooding, rapid seedling growth
DX61	L202	Black Gora	Seedling vigor, rapid growth rate
DX73	L203	Black Gora	
DX128	L202*2	Black Gora	
DX63	M202	IR26702-25	Submergence tolerance
DX131	M204	IR26702-25	
DX133	L203	IR26702-25	
DX72	L203	Azucena	Large panicles, aroma
DX74	L203	CI9858	Good indica type
DX79	M202	CI9858	
DX87	M204	CI9858	
DX75	L203	Initlog Dalag	Good javanica type, large panicles, strong stems
DX88	M204	Initlog Dalag	
DX76	L203	Kinadelaria	Good javanica type
DX89	M204	Kinadelaria	
DX81	M202	Lu Yu 132 Hao	Good indica type
DX90	M204	Lu Yu 132 Hao	
DX82	M202	*O. nivara*	Introgression study using wild species
DX91	M204	Mayyang Khang	Good javanica type
DX92	M204	Silewah	Cold tolerance genes
DX135	L203	Moroberekan	Javanica type, wide compatibility
DX136	L203	Kinanda	Good javanica type
DX137	L203	Pileng Baybay	Good javanica type
DX143	M204	Pileng Baybay	
DX138	L203	Dular	Seedling vigor
DX147	M204	Dular	
DX142	M204	Serendah Kuming	Good javanica type
DX161	M204	IR40931-26	Submergence tolerance

Table 4. Origin of varieties in main cluster groups for RAPD survey of 138 rice varieties.

Origin	Number of cultivars in each group			
	Indica	Japonica 1	Japonica 2	Japonica 3
USA long grain		14		
USA short/medium			6	14
Australia				4
Japan			1	21
Europe	2			29
Korea	2			10
China	3			6
Tropics	9	9	2	3
Wild sp	2			
TOTALS	18	23	11	87

Table 5. Progress in backcrossing components useful for hybrid rice seed production into California-adapted varieties, 1993. cms = cytoplasmic male sterility, R = restoring ability, wc = wide compatibility.

Trait	Cross	Parentage	Comments
cms	DX139	Wu 10A/M202//M204	Chinsurah boro source
	DX141	CMS-RA/2*CM101	Unknown source
	DX152	CMS-RA/2*M202	"
	DX155	CMS-RA/CM101//2*M204	"
	DX162	CMS-RA/3*L203	"
	DX157	IR58025A/3*M204	WA source
	DX159	IR58025A/3*M202	"
	DX153	IR62829A/3*M202	"
	DX156	IR62829A/3*M204	"
R	DX123	M201/IR50R//M103	
	DX127	L202/IR50R//L202	
	DX129	L203/IR50R//L203	
	DX158	M201/IR50R//M201	
wc	DX119	Lemont/2*M202	
	DX120	Lemont/2*M204	
wc/R	DX121	Lemont/IR50R//L203	
	DX122	Lemont/IR50R//M204	

Table 6. Photoperiod-sensitive genetic male sterile mutants developed and characterized at Davis.

Designation	Inheritance	Characteristics	
P1543851	1 recessive	Forms	Original is C76 background from tissue culture-induced mutant. Most lines are late and photoperiod-sensitive like C76 but in 1993, early and glabrous types were isolated.
		Behavior	Very "leaky": shows 10-20% seed set with bagged panicles and about 50% pollen fertility at Davis. Has almost normal fertility in southern US. Is unstable in current background. We are currently making crosses to observe the effect of genetic background.
ST25H/M	2 genes (?)	Forms	Original is M202 background from induced mutation. A diverse population exists developed from open pollinated panicles and some crosses have been made.
		Behavior	Very stable; sterile in California and southern US. Almost 100% pollen sterility. Did not produce seed in winter greenhouse. We are growing it in Hawaii to see if we can produce selfed seed.

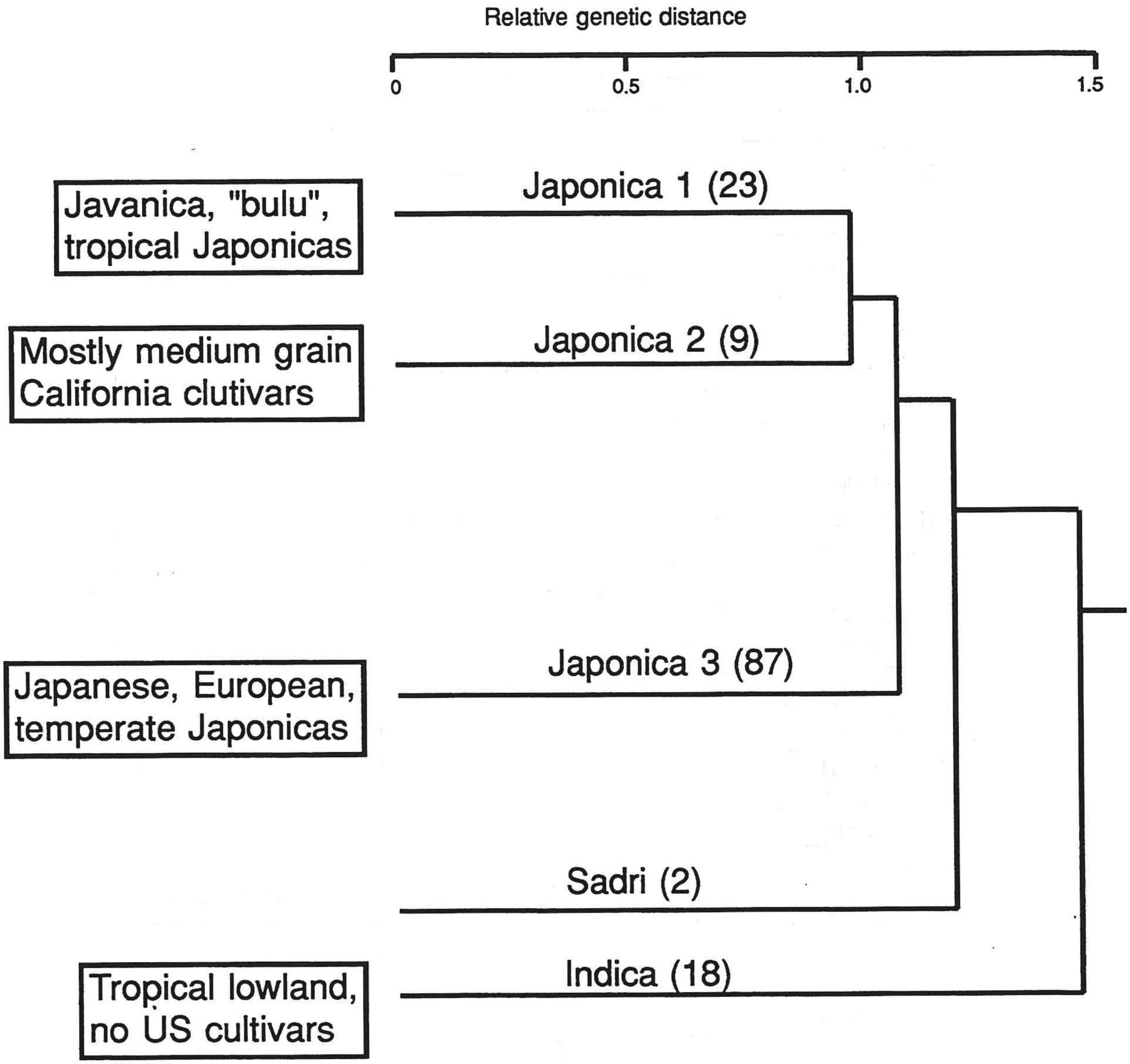

Fig. 1. Summary of cluster analysis for 138 rice varieties based on RAPD analysis using 10 primers and 30 loci. The number in parentheses is the number of varieties in each cluster.

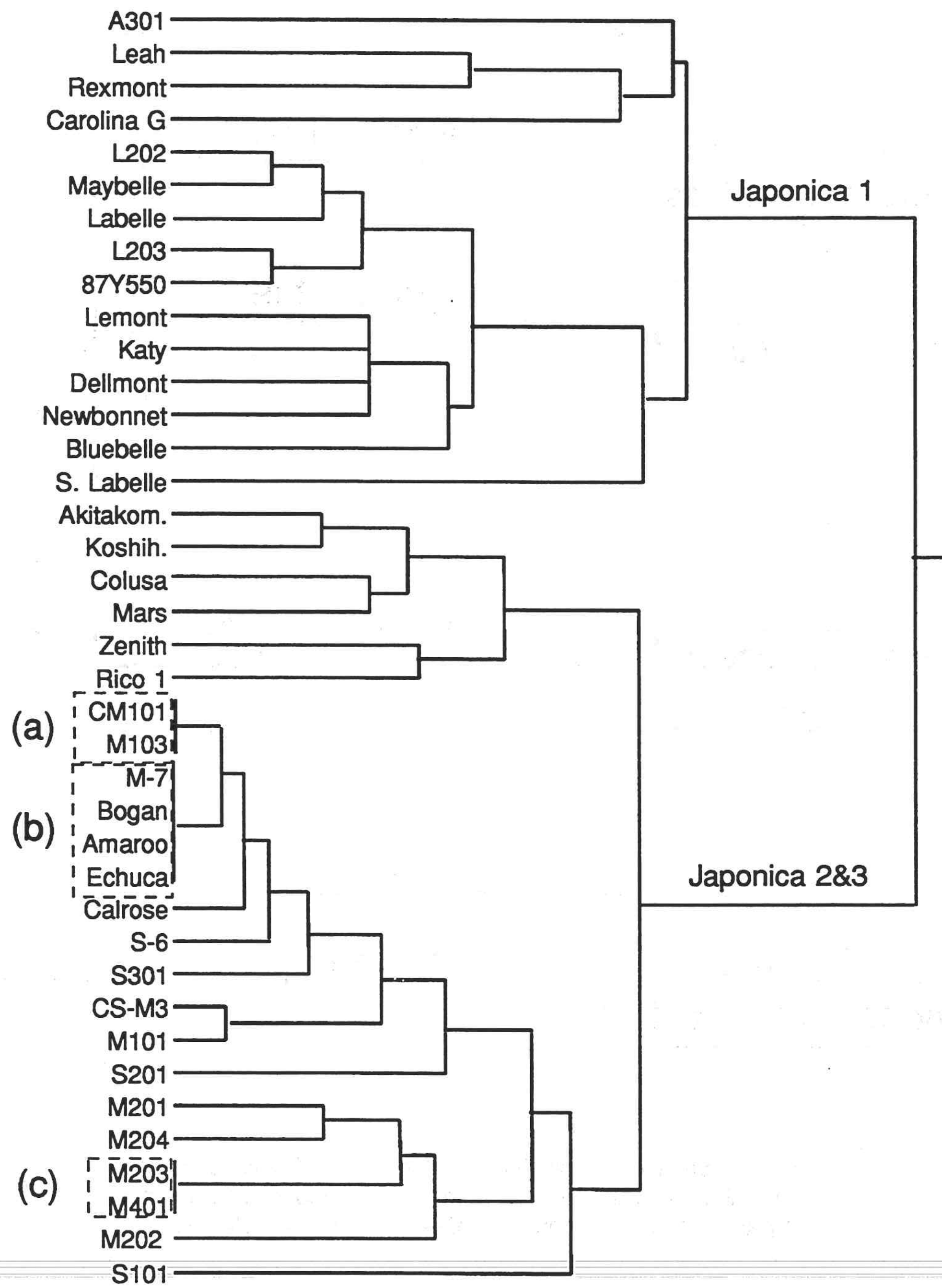

Fig. 2. Cluster analysis of US (and Australian) varieties based on RAPD analysis with 21 primers and 43 loci. Three varietal groups could not be distinguished (a, b, c).