RF 8508 A26

IRRI
Wide Cross

FORM 372

LMH		March 15, 1985

Dear Ralph:

As discussed in our meeting in your office last month and on the telephone, I have enclosed a draft of a proposal that was forwarded to the Foundation by IRRI on "Wide Hybridization for Rice Improvement." We believe that this subject deserves a position in our biotechnology program on cereals, and think IRRI is a logical focus for this research. However, before we recommend a long-term commitment on the part of the Foundation to our Board of Trustees, we will need to refine the rationale behind the research goals and focus the research on those areas with the highest priority. I would like to ask if you would kindly visit IRRI and assist in the refinement of this proposal. We will forward a letter appointing you as a consultant to The Rockefeller Foundation and will provide an honorarium plus full costs, (including additional consultants as needed). With your permission, I shall contact Dr. Swaminathan and inform him of this arrangement.

I would like to offer the following comments for your consideration and advice:

(A) In Attachment A, I have listed selected goals for rice improvement breeding programs in the LDCs and attempted to group them according to the progress that has been made through classical plant breeding. Please look this list over carefully, and give me your comments at your convenience. While new techniques and methodology (through biotechnology) may increase the rate of progress on those topics that are being addressed satifactorily through standard breeding programs, it is likely that the highest priority for the development and application of biotechnology should be on those topics that are perceived as most "critical" and are not being resolved through current breeding programs.

Although sometime labeled differently, the most important criteria for judging the impact of rice improvement programs in the LDCs seem to be productivity, yield stability, yield sustainability, equity, and energy. It is very important for the Foundation to identify the topics that it wishes to give priority and to anticipate the impact that a successful, modern crop improvement program ultimately may have in the LDCs. This question of impact is currently less important for the portion of our program that would deal with transformation of rice through micro injection, vectors, protoplast fusion, etc., since considerable fundamental work has to be accomplished. However, since the time frame for the application of tissue culture and wide cross techniques is considerably shorter, the necessity to identify the

priorities and to anticipate the impact is urgent. Some people suggest that we may see some success within five to ten years in a wide cross program in rice. We need to know what IRRI anticipates will be the ultimate impact of a wide cross program.

You should know that I have asked Randy Barker, Cornell University, to assist us in developing a program concerned with identifying priorities and anticipating the impact that a successful biotechnology program on rice may have in the LDCs. At this early stage in the program, it is difficult to develop an impact study because we cannot be certain which transformation system will ultimately be of practical value. The wide hybridization program at IRRI therefore should be very useful in initiating this component of our program, and should contribute to the development of a rational long-term strategy for the Foundation's cereal biotechnology program.

(B) It would seem appropriate to identify the current state of the art of wide hybridization for rice improvement. It would be helpful to know more about our current ability to make specific crosses, what institutions have a wide hybridization program on rice and their particular goals, what laboratories have an interest and capacity to conduct wide hybridization research on cereals in general, and the status of germplasm collection of wild grassy relatives of rice. We may wish to award grants to certain of these laboratories after conferring with IRRI in order to develop appropriate technology for spcific wide crosses. Incidently, I did discuss this project with Neil Rutger, UC-Davis, who is with the USDA at Davis and is the principal rice geneticist and breeder. Neil is a consultant to the Foundation, and would be willing to assist you if needed and at your convenience.

(C) Once we have a better idea of the goals of the program and the research required in order to accomplish these goals, a new budget will need to be prepared. I suspect that the budget proposed in the IRRI document seriously underestimates the amount of money required for an acceptable program. However, much of the routine screening and evaluation could be done through the IRRI GEU. We may need an estimate of the amount of IRRI's core funds which would be allocated to support this wide cross special project. Our Board of Trustees will be interested in the size of IRRI's contribution to this program, and it will be considered as an indication of IRRI's commitment to this effort.

(D) It would be very helpful to have a little better understanding of the proposed organization and operation of this wide hybridization project at IRRI. From my conversations with M. S. Swaminathan and staff, I am under the impression that a new senior staff member would be needed in the Plant Breeding Department to offer guidance and direction to the program. The ideal candidate would be someone with experience in wide hybridization in cereals or with a background in <u>in vitro</u> studies of morphogenesis and development.

(E) The revised proposal should include a section which deals with the evaluation of the research progress, direction, etc. As you know, the RF program on biotechnology will have a yearly meeting at which time we will bring together the participating scientists. You, of course, are a member of the evaluation panel which will be present at this meeting and will advise the Foundation on the progress of the program future direction, etc. In addition, IRRI does have an annual review and a Technical Advisory Committee review every five years.

We feel very fortunate in having someone with your professional competence and standing assist the Foundation.

With kind personal regards.

Sincerely,

Alva A. App

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AAA:lmh
Encl.

WIDE HYBRIDIZATION FOR RICE IMPROVEMENT

Rice is the major food for more than half of the world's population. In 1982, it was planted to 146 million hectares which produced 435 million tons of grain. More than 95% of the rice is grown and consumed in the developing countries of Asia, Africa and Latin America. The average yields in the developing countries were extremely low. However, substantial increases in rice yields were made after the development and wide scale adoption of short statured, nitrogen responsive and high yielding varieties of rice developed by International Rice Research Institute (IRRI) and by the National Rice Improvement programs. As a result, average yields of rice increased by 50% between 1960-1982.

It is known that only marginal increases in the yield potential of improved varieties have been made over and above the yield potential of first improved cultivar IR8. This is because major attention was paid to stabilize the yield potential of improved varieties through the incorporation of genes for resistance to major diseases and insects. Many multiple disease and insect resistant varieties have now replaced the earlier susceptible dwarfs (Khush, 1984). Wide scale popularity of IR36 is primarily due to its broad spectrum resistance to diseases and insects and tolerance to problem

soils. However, diseases and insects continue to change and develop new races and biotypes. Resistant varieties are thus rendered susceptible.

It is also recognized that the available modern varieties are not suited for growing under unfavorable environments such as rainfed lowland, upland, deep water and tidal wetland situations. Thus the presently available improved varieties are suitable for growing on about 60% the world's rice land.

The problems of rice improvement for the eighties and nineties of this century, therefore are two fold:

(1) Increase the yield potential of the modern dwarfs further and maintain the yield stability through identification and incorporation of diverse genes for host resistance.

(2) Develop improved varieties for the unfavorable environments. Such varieties must have tolerance to adverse situations such as drought, submergence, stagnant flooding and problem soils (salinity, alkalinity, acid sulphate soils, iron toxicity, etc.).

The increases in the yield potential of rice have primarily been achieved through changing the harvest index. The total biomass production has remained the same. Further improvements in the harvest index are unlikely. Therefore further improvements in the yield potential will most likely occur through increases in biomass production (Khush and Virmani, 1984). Therefore, we

must develop genotypes with increased rates of photosynthesis and faster leaf area development. However, within the cultivated varieties of Oryza sativa there seems to be limited variation for these traits. In several other cereal crops such as oats, sorghum and pearl millet, improvements in biomass production and yield potential have been achieved through introgression of wild germplasm into cultivated varieties (Frey, 1983). This approach certainly needs to be tried for breaking the present yield barriers in rice.

Wild species have been successfully exploited to transfer alien genes for disease resistance in important crop species such as wheat and tomato (Knott, 1971; Sears, 1981; Rick, 1982). However, wild germplasm of rice has been utilized to only a limited extent for this purpose. Transfer of grassy stunt resistance gene from O. nivara to O. sativa (Khush, 1977) is the lone example.

Some wild species of rice are highly resistant to major diseases and insects. Several accessions of Oryza australiensis and O. officinalis, for example, are resistant to brown planthopper. Some accessions of O. barthii are resistant to bacterial blight (Table 1). Genes for resistance from wild species if transferred to O. sativa, would help broaden the genetic base for disease and insect resistance and enhance the

the yield stability of improved varieties.

Wild species of rice thrive under poor environments and must have the genetic build up to tolerate adverse conditions such as drought, stagnant floods, and problem soils. O. australiensis thrives under droughty conditions. O. coarctata grows in salty marshes. O. perennis strains are known to thrive in acid sulphate soils (Table 1). These wild species are thus potential reservoirs of germplasm for improving the rices for unfavorable environments.

O. perennis has been successfully used for developing cytoplasmic male sterile lines (Lin and Yuan, 1980) which are now widely used in the hybrid rice breeding programs. However, only one source of cytoplasmic male sterility is used for producing hybrids which were planted to 7 million hectares of rice land in 1983. This narrow base is a potential source of vulnerability of rice crop to diseases and insect epidemics. Alternate sources of cytoplasmic male sterility must therefore be located. Wild species are the most logical sources to investigate for this purpose.

Several morphological traits which are of immense value in the hybrid rice program such as long anthers, long and protruding stigmas with longer receptivity are only present in the wild species and can be transferred to restorer and maintainer lines respectively.

Wide crosses in barley (Hordeum vulgare/H. bulbosum) have been successfully used to produce haploids on mass scale (Kasha and Kao, 1970) and variety Mingo developed through haploid breeding technique has been released. Wild species of rice need to be explored for their ability to produce haploids when used as pollen parent in crosses with cultivated rice.

Before embarking upon a program of gene transfer from wild into cultivated species, basic information about crossability, genome relationships and amount of intergenomic recombination if any is very useful. Unlike wheat where genomic relationships of many wild species with those of cultivated wheat have been thoroughly investigated, the genomic relationships of species of Oryza are imperfectly understood. The present status of our understanding of genomic relationships of various species of Oryza are shown in table 2. It is evident that many gaps in our understanding of species relationships in Oryza exist.

Objectives of the project

The major objectives of the project would be as follows:

(1) To explore the possibility of increasing the biomass and yield potential of rice through introgression of genes

from wild into cultivated species.

(2) To transfer alien genes for resistance to major diseases and insects from wild into cultivated rice.

(3) To incorporate genes for stress tolerance (drought, submergence, stagnant flooding, problem soils) from wild in cultivated species.

(4) To develop an array of cytoplasmic male sterile lines with diverse cytoplasms, for use in hybrid rice breeding.

(5) To accumulate basic information about the species relationships in Oryza such as crossabilities, genome homologies, genetic control of chromosome pairing, mechanisms of chromosome differentiation and nucleo-cytoplasmic relationships.

Plan of Work

Some or all of the following studies will be undertaken.

(1) Evaluation of the wild germplasm for economic traits.

Some accessions of wild species have been evaluated for economic traits. Remaining wild germplasm will be screened in cooperation with scientists in various disciplines.

(2) Production of interspecific hybrids and extraction of stable derivatives.

The wild species showing useful genetic variability will be crossed with elite varieties or breeding lines to

produce interspecific hybrids. Techniques of embryo resuce, in-vitro fertilization and protoplast fusion will be used where necessary to overcome the crossability barriers.

The F_1 combinations showing reasonable fertility will be backcrossed two or three times to the cultivated parent to derive breeding lines with traits of economic importance such as higher biomass production, disease or insect resistance, stress tolerance or morphological or cytoplasmic traits of usefulness in hybrid rice breeding.

The hybrids showing lack of chromosome pairing and recombination will be utilized to derive alien addition or substitution lines. These will be screened to identify the alien chromosomes responsible for traits of economic importance and efforts will be made to transfer chromosome segments bearing these traits to the genome of cultivated rice through radiation treatments.

(3) Characterization of nuclear cytoplasmic relationships.

Crosses of wild species with cultivated rice will be carefully studied to identify combinations showing cytoplasmic male sterility. Combinations showing this useful attribute will be utilized in the on-going program on hybrid rice breeding.

(4) Production of haploids through wide crosses

Various interspecific crosses will be carefully examined to identify a species if any which when used as a pollen parent might yield haploid progenies like the bulbosum system in barley.

(5) Study of genome relationships

Chromosome pairing in the interspecific hybrids will be examined to determine the genome homologies. These studies will be supplemented by detailed pachytene analysis, and banding patterns after giemsa staining. Genomic relationships will also be studied through in-situ DNA hybridization techniques where labelled DNA probes of O. sativa would be used for hybridization with those of wild species.

(6) Genetic engineering

Possibilities of transferring alien genes into cultivated rice through techniques of molecular biology such as microinjection of alien DNA into microspores and egg cells of cultivated rice, and transformation through pollination of cultivated rice with pollen soaked in alien DNA would be tried. Similarly fusion of unirradiated

protoplasts with irradiated protoplasts would be attempted for limited gene transfer including speedy transfer of cytoplasm and development of male sterile lines (Swaminathan, 1982).

Collaboration with advanced research centers

Research efforts at IRRI will be linked with selected laboratories in advanced countries and research leaders in these laboratories will be invited as consultants. Collaboration with the University of Guelph, Ontario (Canada) where pioneer work on widecrosses has been done, will be sought. Similarly collaboration with Plant Breeding Institute Cambridge (U.K.) where extensive work on wide hybridization in cereals is underway will be established.

Facilities, manpower and materials available

IRRI has an excellent collection of wild species of rice in its germplasm bank and has contacts with national program scientists for collecting more wild germplasm. Well equipped cytogenetics laboratory, green and screenhouses and field facilities are available. Facilities of tissue culture laboratory are available for embryo rescue and in-vitro fertilization work. Various facilities for evaluating the products of wide crosses for traits such as disease and insect resistance, and

stress tolerance are available within the GEU program at IRRI. Progenies with useful traits will be immediately utilized by the breeders working on rice improvement for various cultural types and hybrid rice. The materials will also be shared with interested breeders working National Rice Improvement programs through IRRI's network activities.

Facilities and manpower needed

A full time scientist with basic training in cytogenetics but with broad interest and experience in areas of biotechnology such as tissue culture, protoplast fusion, gene transfer techniques and keen interest in crop improvement is needed to serve as leader of this project. He should be assisted by one post-doctoral, two research assistants and other support staff.

Duration of the project

It is essential that the project is funded for a minimum of five years but a duration of ten years is necessary to get meaningful results.

Budgetary needs

The budgetary needs for the project which are primarily for the staff salaries and supplies are shown in appendix I.

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Table 1. Chromosome number, genome symbol, and geographical distribution of *Oryza* species.

Section, Species	2n	Genome	Geographical distribution
Section *Oryzae*			
sativa L.	24	AA	Worldwide, cultivated
rupifogon Griff (=perennis Moench)	24	AA	Asia, America
barthii A. Chev.[a] (=longistaminata A. Chev. et Roehr)	24	AA	Africa
	24	AA	
glaberrima Steud.	24	AA	Africa, cultivated
breviligulata A. Chev et Roehr (=*barthii* in the sense of Clayton, 1968)	24	AA	Africa
australiensis Domin	24	EE	Australia
eichingeri A. Peter	24	CC	Africa
punctata Kotschy	24,48	BB, BBCC	Africa
officinalis Wall	24	CC	Asia
minuta J. S. Presl.	48	BBCC	Asia
latifolia Desv.	48	CCDD	America
alta Swallen	48	CCDD	America
grandiglumis Prod.	48	CCDD	America
Section Schlechterianae			
shlechteri Pilger		?	New Guinea
Section Granulatae			
meyeriana Baill. (-granulata Nees et Arn.)	24	?	Asia
Section Ridleyanae			
ridleyi Hook, f	48	?	Asia
longiglumis Jansen	48	?	New Guinea
Section Angustioliae			
brachyantha A. Chev. et Roehr.	24	FF	Africa
angustifolia Hubbard	24	?	Africa
perrieri A. Camus	24	?	Malagasy
tisseranti A. Chev.	24	?	Africa
Section Coarctatae			
coartata Roxb.	48	?	Asia

[1] From Morishima (1984)

Table 2. Some wild species of *Oryza* with traits of economic importance.

Wild species	Useful trait
O. nivara	Resistance to grassy stunt and blast
O. barthii	Resistance to bacterial blight
O. longistaminata	Floral characters for outcrossing
O. glaberrima	Resistance to green leafhopper
O. officinalis	Resistance to brown planthopper
O. australiensis	Resistance to brown planthopper and drought
O. coarctata	Resistance to salinity
O. perennis	Tolerance to acid sulphate soils and stagnant flooding. Source of sterile cytoplasm.

Proposed Budget for
Wide Hybridization for Rice Improvement
For a five-year period
(in US $)

	Year 1	Year 2	Year 3	Year 4	Year 5	Total
1. Salaries and Benefits						
a. Senior Staff (1 position/ 5 man-years)	$ 84,900	$ 75,100	$ 82,700	$104,000	$103,400	$ 450,100
b. Support Staff Research Assistants - 2 positions/10 man-years) Laborers - 5 positions/ 25 man-years)	11,800	13,000	14,300	15,700	17,300	72,100
c. Post-Doctoral Fellow	24,600	27,400	29,700	28,800	36,200	146,700
Sub-total	$121,300	$115,500	$126,700	$148,500	$156,900	$ 668,900
2. Short-term Consultant	$ 10,200	$ 11,200	$ 12,300	$ 10,800	$ 11,900	$ 56,400
3. Equipment and Supplies	$ 15,000	$ 16,500	$ 18,200	$ 20,000	$ 22,000	$ 91,700
4. Travel						
a. International	$ 4,400	$ 4,900	$ 5,400	$ 6,000	$ 6,600	$ 27,300
b. Local	1,000	1,100	1,200	1,300	1,400	6,000
Sub-total	$ 5,400	$ 6,000	$ 6,600	$ 7,300	$ 8,000	$ 33,300
5. Indirect Cost	$ 46,300	$ 45,500	$ 50,000	$ 56,900	$ 60,600	$ 259,300
T o t a l	$198,200	$194,700	$213,800	$243,500	$259,400	$1,109,600

/s
10/9/84

CAPACITY OF TRADITIONAL BREEDING PROGRAMS TO RESOLVE CONSTRAINTS ON RICE YIELDS

Biomass Production
Drought Tolerance
Cold Tolerance
Salinity Tolerance

Bacterial L.B. Resistance
Blast Resistance
Hopper Resistance
Stemborers Resistance
Male Sterility
Nutrition Deficiency Tolerance
Problem Soils Tolerance
Grassy Stunt Resistance
Tungro Resistance
Ragged Stunt Resistance

Grain Quality
Pesticide Tolerance

Crop Duration
Dwarfism
Photoperiodism

Transfer of Specific Chromosomes from Wild Species of *Oryza* into Cultivated Rice

(A progress report by Dr. K. K. Jena)

(PDF at IRRI)

Several wild species of rice are highly resistant to diseases and insects of rice. It is easy to transfer useful genes from the wild species with AA genome to cultivated rice. For example, grassy stunt resistance gene *Gs* was transferred from *O. nivara* ~~to~~ to cultivated rice through conventional hybridization approach. However, wild species whose genomes are non-homologous to the A genome of *O. sativa* are extremely difficult to cross with cultivated rice. Their genomes do not pair and recombine with A genome. Therefore, it is extremely difficult to transfer genes from these species into cultivated rice.

This study was undertaken to determine the possibility of establishing alien monosomic addition lines having full chromosome complement of *O. sativa* and single chromosomes of the wild species. Segments of alien chromosomes bearing the useful gene would then be transferred to the A genome by radiation treatments.

Materials and Methods:

For this purpose 18 accessions of *O. officinalis* (CC genome) and 4 of *O. australiensis* (EE genome) were obtained (Table 1). These accessions are resistant to all the known biotypes of brown planthopper (BPH) -- a most serious pest of rice. These accessions

were crossed with three breeding lines of O. sativa (Table 1). The breeding lines are susceptible to BPH.

A number of crosses between O. sativa lines as females and wild species as males were attempted. The range of seed set was 0.5% to 30.86%. However, the seeds were imperfectly developed and started to abort 14 days after pollination. Examination of the aborting seeds revealed that the embryo development was normal and the abortion was due to degeneration of the endosperm. To overcome this problem 14 days old embryos were excised under a stereo microscope in an aseptic condition and were cultured on 1/4 MS medium devoid of auxin. The cultured embryos were incubated in dark until germination and subsequently were transferred to light incubation room. The rate of embryo germination varied from 33.33 to 88.20% in different combinations (Tables 2-4). The young seedlings at three leaf stage were transferred to liquid nutrient solution. After 10 days, the seedlings were transferred to soil. This way more than 450 interspecific hybrid plants have been obtained.

The F_1 plants are intermediate between the two parents in some respects but they express several traits of wild parents. In general, they are robust and extremely vigorous. However, they are completely male sterile.

Cytology of F_1 hybrids:

A vast majority of the F_1 plants are diploid. However, 5 F_1 hybrids from a cross of IR1529-680-3-2 and O. officinalis (Acc. No. 100179) turned out to triploid. The modal chromosome pairing in diploid hybrids was 4 bivalents with a range of 0 to 10. In the triploid hybrids the range of bivalents during meiosis I was 5 to 7. These triploids probably resulted from the pollination of unreduced gametes of one parent and should have two homologous genomes. However, lower frequency of bivalents suggests that the presence of pairing suppresser genes in one of the genomes.

Backcross progenies:

The diploid as well as triploid hybrids have been backcrossed using breeding lines of O. sativa as recurrent parents. The seed set was extremely low, the percentage being 0-5.2% in triploid hybrids and 0-8.58% in diploid hybrids. Nevertheless, a few well formed seeds were obtained which have germinated to produce healthy seedlings. Some of these backcross progenies especially those from triploid hybrids are expected to be monosomic alien addition lines having diploid chromosome complement of O. sativa and single alien chromosomes of O. officinalis or O. australiensis.

Future plans:

The backcross progenies will be cytologically examined to determine their chromosome number. Detailed analysis of these progenies will be carried out to find their male and female fertility and presence of any traits introduced from the wild species. Each of the alien addition lines will be backcrossed to O. sativa. The BC_2 progenies will be evaluated for resistance to BPH to identify the alien chromosome or chromosomes which carry the genes for resistance. Efforts will be then made to transfer the genes for BPH resistance from these alien chromosomes to the genome of O. sativa either through the rare recombinational events or through radiation treatments.

Triploid hybrids are good sources of monosomic alien addition lines. However, up to now, triploid hybrids of O. sativa and O. australiensis have not been obtained. Colchicine induced tetraploids of O. sativa have been produced. These will be pollinated with diploid O. australiensis to obtain triploid hybrids.

Table 1. Cultivated and wild species of rice used in the study.

Name of species	Acc. No.	Country of origin	Chromosome No.	Genome	Reaction* to BPH
O. officinalis	100179	Japan	24	CC	R
O. officinalis	100180	Malaya	24	CC	R
O. officinalis	101121	Philippines	24	CC	R
O. officinalis	101113	Philippines	24	CC	R
O. officinalis	101117	Philippines	24	CC	R
O. officinalis	101077	Philippines	24	CC	R
O. officinalis	101078	Philippines	24	CC	R
O. officinalis	101150	Malaysia	24	CC	R
O. officinalis	101152	Malaysia	24	CC	R
O. officinalis	101155	Malaysia	24	CC	R
O. officinalis	102385	Indonesia	24	CC	R
O. officinalis	102382	Indonesia	24	CC	R
O. officinalis	101414	India	24	CC	R
O. officinalis	101412	India	24	CC	R
O. officinalis	100947	India	24	CC	R
O. officinalis	100896	Thailand	24	CC	R
O. officinalis	100878	Thailand	24	CC	R
O. officinalis	101399	Vietnam	24	CC	R
O. australiensis	100882	India	24	EE	R
O. australiensis	101144	Australia	24	EE	R

Table 1. (contd.)

Name of species	Acc. No.	Country of origin	Chromosome No.	Genome	Reaction* to BPH
O. australiensis	101397	USA (USDA)	24	EE	R
O. australiensis	101410	Australia	24	EE	R
O. sativa (IR1529-680-3-2)		IRRI	24	AA	S
O. sativa (IR25587-109-3-3-3-3)		IRRI	24	AA	S
O. sativa (IR31917-45-3-2)		IRRI	24	AA	S

* R = Resistant; S = Susceptible.

Table 2. Crossability and embryo rescue success rate in crosses of O. sativa (IR1529-680-3-2) with wild species.

Wild species Acc. No.	Spikelets pollinated (No.)	Seed set (No.)	Seed set (%)	Embryos cultured (No.)	Embryos germinated (No.)	Embryos germinated (%)	True hybrids obtained (No.)	Crossability (No.)
100179	2919	257	8.80	148	83	56.08	50	1.71
100180	1821	246	13.45	174	78	44.82	50	2.74
101121	2331	168	7.20	98	72	73.46	36	1.54
101113	59	6	10.16	6	3	50.00	3	5.08
101150	300	30	10.00	16	11	68.75	4	1.33
101414	487	6	1.23	5	5	100.00	2	0.41
100896	183	2	1.09	2	2	100.00	1	0.54
100882	1958	49	2.50	34	13	38.23	12	0.61
101397	1378	36	2.61	36	27	75.00	22	1.59
101144	2014	38	1.88	27	21	77.77	12	0.59

Table 3. Crossability and embryo rescue success rate in crosses of O. sativa (IR25587-109-3-3-3-3) with wild species.

Wild species Acc. No.	Spikelets pollinated (No.)	Seed set (No.)	Seed set (%)	Embryos cultured (No.)	Embryos germinated (No.)	Embryos germinated (%)	True hybrids obtained (No.)	Crossability (%)
100179	1226	113	9.21	41	21	51.21	13	1.06
100180	1413	169	11.96	19	12	63.15	5	0.35
101121	687	91	13.24	45	28	62.22	15	2.18
101113	193	24	12.43	13	6	46.15	1	0.51
101117	677	55	8.12	4	4	100.00	4	0.59
101077	110	4	3.63	1	1	100.00	1	0.90
101078	173	33	19.07	15	13	86.66	13	7.51
101150	734	80	10.89	15	5	33.33	4	0.54
101152	670	83	12.38	12	5	41.66	-	-
101155	778	65	8.35	18	11	61.11	7	0.89
102385	863	30	3.47	5	3	60.00	2	0.23
102382	535	29	5.42	15	12	80.00	11	2.05
101414	242	8	3.30	1	1	100.00	-	-
101412	264	27	10.22	13	9	69.23	5	1.89
100947	664	68	10.24	17	15	88.20	7	1.05
100896	352	37	10.51	3	0	-	-	-
100878	225	33	14.66	14	11	78.57	5	2.22
101399	333	68	20.42	15	12	80.00	9	2.70
100882	394	10	2.53	5	4	80.00	3	0.76
101397	984	31	3.15	7	5	71.42	3	0.34
101144	1377	36	2.60	8	4	50.00	1	0.07
101410	769	4	0.52	12	5	41.66	2	0.26

Table 4. Crossability and embryo rescue success rate in crosses of O. sativa (IR31917-45-3-2) with wild species.

Wild species Acc. No.	Spikelets pollinated (No.)	Seed set (No.)	Seed set (%)	Embryos cultured (No.)	Embryos germinated (No.)	Embryos germinated (%)	True hybrids obtained (No.)	Crossability (%)
100179	1896	352	18.56	59	39	66.10	28	1.47
100180	871	225	25.80	71	49	69.01	36	4.13
101121	79	16	20.25	15	12	80.00	12	15.18
102385	569	51	8.96	8	5	62.50	4	0.70
101117	1192	181	15.18	36	31	86.11	16	1.34
101150	285	12	4.21	9	7	77.77	6	2.10
101113	558	84	15.0	22	14	63.63	9	1.61
101152	476	102	21.42	20	14	70.00	5	1.05
100896	408	71	17.40	28	21	75.00	20	4.90
100878	169	30	1.77	19	10	52.63	7	4.14
101414	189	28	14.81	22	13	59.09	9	4.76
101155	294	57	19.38	8	5	62.50	5	1.70
102382	70	8	11.42	8	5	62.50	1	1.42
101412	197	26	13.19	9	9	100.00	5	2.53
101077	81	25	30.86	18	15	83.33	11	13.58
101399	297	62	20.87	13	8	61.50	6	2.02
101078	154	17	11.03	9	5	55.55	3	1.94
100882	694	27	3.89	14	6	42.85	3	0.43
101397	738	20	2.71	3	3	100.00	3	0.40
101144	840	26	3.09	16	8	50.00	3	0.35
101410	800	14	1.75	9	5	55.55	4	0.50

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Rice Improvement by Wide Hybridization and by the International Coordination of Rice Genetics

PROGRESS REPORT

RICE IMPROVEMENT BY WIDE HYBRIDIZATION

The Wide Hybridization project was started five years ago with the objective of transferring desirable traits from the wild Oryza species into cultivated rice (O. sativa) and to generate new variability for use in rice improvement.

Progress during 1987/1988

1. Selection of insect resistant O. officinalis-derived progenies

In 1984, crosses were made between three elite O. sativa (AA) lines (susceptible to brown planthopper (BPH) and white-backed planthopper (WBPH)) and 18 O. officinalis (CC) accessions (resistant to BPH and WBPH). The hybrids were backcrossed and fertile progeny selected.

In 1987, 103 O. officinalis-derived lines were identified with resistance to BPH biotypes 1, 2, and 3 and of these, 50 were also resistant to the Mindanao biotype. In addition, 202 lines were found to be resistant WBPH. Lines from this cross also performed well in the replicated yield trial. The BPH and WBPH resistant lines have been sent for field evaluation in Indonesia, Malaysia, Vietnam, Bangladesh and India. Enough seeds of these lines have been produced for inclusion in 1989 IRTP nurseries.

2. Transfer of BPH and WBPH resistance from O. minuta

O. minuta (BBCC) is a rich source of resistance to plant and leafhoppers. We crossed IR31917-45-3-2 (susceptible to BPH and WBPH) with four Philippine accessions of O. minuta (Table 1). The 18 triploid (ABC) F_1 hybrids obtained from crosses with 101089 and 101141 were highly sterile, giving only seven small shrivelled BC_1 seeds. Following embryo rescue, four plants were obtained, two of which died probably due to chromosomal abnormalities. The remaining two plants are tetraploid (AABC) and are being used for BC_2 production.

An attempt was made to improve hybrid fertility by doubling their chromosome number through colchicine treatment. Nine partially-fertile colchicine-treated plants were obtained and used to produce a further 12 BC_1 seeds. Of the nine embryos extracted, four germinated, increasing the number of BC_1 plants available to six.

IR31917-45-3-2 and O. minuta (Acc. 101089, 101141) also differ in their resistance to blast and bacterial blight. All F_1 hybrids were resistant to blast. The hybrids derived from 101141 were resistant to all six Philippine races of the bacterial blight pathogen. Those from 101089 showed varying levels of resistance, suggesting that this accession is heterozygous for the resistance locus/loci. (This work is being done as part of Masters thesis project, jointly supervised with the Plant Pathology Department).

3. Transfer of BPH resistance from O. eichingeri

In 1985, crosses between IR31917-45-3-2 and BPH resistant O. eichingeri (Acc. 101421; CC) produced diploid F_1 hybrids. Subsequent backcrossing produced 10 BC_1 plants and, in 1987, two BC_2 plants. These BC_2 plants will be advanced further to produce addition lines and to transfer BPH resistance from O. eichingeri into O. sativa.

4. Transfer of BPH resistance and drought tolerance from O. australiensis

In 1985, crosses between IR31917-45-3-2 and O. australiensis (Acc. 100882; EE) produced a number of diploid hybrids. However, backcrossing was unsuccessful. A tetraploid form of IR31917-45-3-2 was then produced by colchicine treatment and hybridized with Acc. 100882. Subsequent backcrossing produced 16 BC_1 plants with chromosome numbers ranging from 28-31. In 1987, approximately 30 BC_2 seedlings were produced; their chromosome numbers will be determined later.

5. Interspecific hybridization as a tool for increasing the yield potential of rice

Two approaches were adopted to evaluate the potential of the wild species for increasing the yield potential of rice.

(i) O. sativa × O. latifolia (CCDD)

O. latifolia is a tall, broad-leaved species with a high biomass. We crossed O. sativa (IR36, IR64) and four O. latifolia accessions and produced 101 F_1 hybrids (Table 2). These plants were highly sterile. After considerable backcrossing, four BC_1 plants were obtained. The restoration of hybrid fertility through colchicine treatment was unsuccessful.

(ii) O. sativa x AA genome species

Frey and co-workers demonstrated that the yield potential of oats, barley and sorghum can be increased through the introgression of genes from weedy relatives which are genetically closely related to the cultivated species.

A similar backcrossing and selfing program is underway with the aim of introgressing genes from O. nivara and O. rufipogon into cultivated rice. The hybrids show normal chromosome pairing and therefore the introgression of minor genes influencing quantitatively-controlled traits may be expected.

6. Identification of new sources of cytoplasmic male sterility

Reciprocal crosses between two O. sativa restorer lines (IR54R, IR64R) and 36 randomly selected O. rufipogon and "O. perennis" accessions were made. Initially, pollen fertility was determined on hybrids derived from five accessions. One O. rufipogon/O. sativa hybrid and three "O. perennis"/O. sativa hybrids were identified with less than 30% pollen fertility. These hybrids were backcrossed with O. sativa and seeds obtained from three hybrids (Table 3). Hybrids derived from crosses with the remaining 31 accessions are being grown for evaluation.

7. O. rufipogon as a source of elongation ability and stem borer resistance.

In August 1987, hybrids between eight O. sativa genotypes and an O. rufipogon accession from Bangladesh, selected for its good elongation ability, were given to the project by the Deepwater rice program. Since then BC_1, BC_1F_2, and BC_2 progeny have been produced. In some crosses embryo rescue was needed to ensure survival of the backcrossed seeds.

The elongation ability of the parents and hybrids is currently being evaluated in collaboration with the Plant Physiology Department.

The O. rufipogon parent and the hybrids showed a high level of resistance to stem borers when tested by the Entomology Department. All parents and hybrids are being retested and the backcross-derived populations will be evaluated later.

8. New interspecific hybrids

Additional interspecific crosses were made with the following objectives:

(i) to create new hybrids with O. sativa.

Crosses between O. sativa (IR64) and O. grandiglumis (Acc. 101405; CCDD) gave an average of 6.7% seed set. All 22 hybrid seeds were poorly developed. Thirteen embryos were extracted and seven plants obtained. These are still at the vegetative stage.

O. brachyantha (FF), a source of resistance to stem borer and rice whorl maggot, was extensively crossed with O. sativa, using O. brachyantha as either the male or female parent. All 18 seeds were poorly developed; 12 embryos were extracted. The vegetative apearrance of the three O. sativa/O. brachyantha hybrids is similar to that of O. brachyantha.

(ii) to identify the genomic constitution of O. granulata, O. longiglumis and O. ridleyi.

Crosses were made between these three species and other species of different known genomes. No hybrids were produced. However, seeds were obtained from some crosses involving O. granulata. This work will continue.

9. Performance of O. glaberrima under upland conditions

Upland rice is often exposed to adverse soil conditions, such as phosphorous deficiency. O. glaberrima is often cultivated under upland conditions and may therefore be a source of tolerance to such adverse soil conditions. During the 1987 Dry and Wet Seasons, O. glaberrima accessions were screened in two phosphorous-deficient upland trial sites. Very few accessions showed any tolerance to the poor conditions. However, genotypic variation for blast resistance was observed and certain accessions showed earlier vegetative vigour and a higher tillering ability than the tolerant O. sativa checks. The greater vigour of these accessions may be studied further under controlled conditions.

10. Further evaluation of the wild species for disease and insect resistance

(i) Bacterial blight

As part of a Masters thesis project, 42 accessions of O. longistamainata, O. nivara, "O. perennis" and O. latifolia were tested for resistance to Philippine races of the bacterial blight pathogen. "O. perennis" Acc. 104796 was resistant to all six races and O. nivara Acc. 102166 to races 1 to 5. Other accessions either showed differential responses to the different races or were susceptible to all races. Resistance appears to be controlled by major genes. Crosses will be made between selected accessions to study the genetics of resistance and further accessions will be screened.

(ii) Sheath blight

A number of wild species were screened for sheath blight resistance. All accessions were susceptible. Many accessions lodged, which presumably influenced the development of the epidemic and hence the results obtained. The experimental procedure will be modified accordingly when further tests are made.

(iii) Rice Tungro Virus

All available wild species accessions were screened by Plant Pathology for resistance to rice tungro virus. Although 62 accessions showed a low level of infection, 44 of these were resistant to green leafhopper, the vector. The low levels of virus infection shown by these accessions could therefore reflect a low virus transmission rate and not resistance to the virus. The majority of these accessions possessed the BB, BBCC, CCDD and EE genomes. However, the remaining accessions, all of which were AA genome species, were susceptible to GLH. These accessions will be retested for virus resistance.

(iv) Insect resistance

Many accessions of the wild species have been tested by Entomology for resistance to GLH, BPH, WBPH, rice whorl maggot, and stem borer. This has added further to the information available on the potential of the wild species as donors of agronomically important characteristics.

Personnel

Dr. L. A. Sitch	Senior Scientist
Dr. K. K. Jena	Postdoctoral Fellow
Ms. Regina Dalmacio	Senior Research Assistant
Ms. Ruth Elloran	Research Assistant
Mr. Gabriel Romero	Research Assistant (joined 1 May 1988)

RICE GENETICS

On-going genetic studies have been considerably expanded and streamlined. Emphasis continues to be on the collection and maintenance of gene marker stocks, preparation of cytological, genetic and molecular maps and promotion of international collaboration in rice genetics through the Rice Genetics Cooperative.

Progress during 1987/1988

1. Collection and maintenance of gene marker stocks.

More than 350 mutant stocks have been assembled and are being maintained at IRRI. Many of the markers are in a japonica background and are thus difficult to multiply at IRRI. We are systematically transferring these mutant genes to the genetic background of IR36 by backcrossing. Of these, about 80 have already been transferred.

2. Chromosomal location of new marker genes.

New mutant genes are evaluated for allelism with the known genes with similar phenotypes. As soon as a non-allelic gene is identified it is crossed with all the primary trisomics to determine its chromosome location. We conducted allele tests with 28 new markers and found 12 new loci. We determined the chromosomal location of few of them. A white panicle mutant was located on chromosome 7, a brittle-noded gene on chromosome 2, a chlorina gene on chromosome 4 and zebra marker on chromosome 1.

3. Chromosomal location of isozyme loci.

Our on-going project on isozyme loci aims to map all the known isozyme loci to respective chromosomes and determine their linkage relations with genes for traits of economic importance such as disease and insect resistance. Close association if any between such genes and isozyme loci will be helpful in tagging some of the genes. This information will be useful in rice breeding and selection programs. During the year, we associated ten isozyme loci with respective chromosomes through trisomic analysis. So far 18 isozyme loci have been associated with 8 of the twelve rice chromosomes (Table 4). We are now in the process of making crosses between the trisomics and Got-2, Got-3, Pgd-2, Mal-1, Acp-4, Enp-1, Mdh-1, Mdh-2 and Mdh-3, to determine chromosomal locations of these markers.

4. RFLP maps

In cooperation with Cornell University, an RFLP map consisting of 120 DNA markers was prepared. For this purpose seed materials and lypholized leaf tissues of segregating populations and primary trisomics were regularly sent to Cornell. The second phase of this project involves determination of linkages if any between the RFLP loci and genes for traits of economic importance such as disease and insect resistance or drought tolerance. An F_2 population from a cross specifically produced for this purpose were grown and lypholized leaf tissues from each plant were sent to Cornell. F_3 lines are being evaluated at IRRI for disease and insect resistance and the data will be used for linkage analysis. New crosses have been designed.

5. Cytological maps

The cytological maps of rice are still not well known. The position of centromeres on the pachytene chromosome map are still uncertain. We are standardizing the techniques of chromosome banding (N and C banding) and in-situ hybridization to identify each chromosome precisely.

6. Search for transposable elements.

We have observed considerable instability in the breeding lines derived from crosses of O. sativa and O. officinalis. Unstable waxy mutants are being characterized in collaboration with Susan Wessler of University of Georgia and anthocyanin mutants in collaboration with Virginia Walbot of Stanford University.

7. Genetics of disease and insect resistance

The allelic relationships of genes for BPH and WBPH resistance obtained from O. officinalis with those already

known, are being determined. A gene for bacterial blight resistance from O. longistaminata is similarly being analysed.

8. Rice Genetics Cooperative

A meeting of the Coordinating Committee of Rice Genetic Cooperative was held at IRRI on October 22-24, 1987. It was decided to hold the 2nd International Rice Genetic Symposium in May 1990 and an International Organizing Committee was established. Rules for the gene symbolization proposed in Rice Genetics Newsletter Volume 3 were approved.

9. Rice Genetics Newsletter

Rice Genetics Newsletter Vol. 4 was published. It contains a report of the committee on gene symbolization, list of new genes, current linkage map, list of genes being maintained at Rice Genetic Stock Center at IRRI and 32 research notes.

Personnel

Dr. G. S. Khush	Principal Plant Breeder
Dr. D. S. Brar	Associate Plant Breeder
Ms. Alma Cruz	Research Assistant
Mr. Benildo delos Reyes	Research Aide

Table 1. **Seed set and embryo rescue data from crosses of O. sativa (IR31917-45-3-2) with selected O. minuta accessions.**

O. minuta Accession No.	Spikelets Pollinated (No.)	Seed Set No.	Seed Set %	Embryos Cultured (No.)	Embryos Germinated No.	Embryos Germinated %	Hybrid Obtained (No.)
101089	529	16	3.0	10	6	60.0	4
101096	1117	43	3.8	1	0	-	-
101099	213	0	0	0	-	-	-
101141	1553	66	4.2	29	14	48.3	14

Table 2. **Seed set and embryo rescue data from crosses of *O. sativa* (IR36, IR64) with selected *O. latifolia* accessions.**

Cross combination		Spikelets Pollinated (No.)	Seed Set		Embryos Cultured (No.)	Germinated	
			No.	%		No.	%
IR36 x Acc. No.	100963	254	11	4.3	10	7	70.0
	100964	413	4	0.1	4	0	0
	100965	430	4	0.1	4	0	0
	100966	454	33	7.3	28	25	89.3
IR64 x Acc. No.	100963	501	26	5.2	24	15	62.5
	100964	269	0	0	-	-	-
	100965	429	0	0	-	-	-
	100966	472	93	19.7	69*	54	78.3

* Embryos extracted from only 78 of the total 93 seeds.

Table 3. **Seed sets obtained from backcrosses of selected *O. rufipogon*/*O. sativa* and "*O. perennis*"/*O. sativa* hybrids (i)**

Hybrid description	Hybrid Fertility (%)	Backcross Parent	Spikelets Pollinated (No.)	Seed Set No.	Seed Set %
O. rufipogon (Acc. 100907) / IR54R	0	IR54	76	17	22.4
"*O. perennis*" (Acc. 104400) / IR54R	11-30	IR54	184	15	8.2
"*O. perennis*" (Acc. 101186) / IR64	0	IR64	124	0	0
		IR64R	45	0	0
"*O. perennis*" (Acc. 104859) / IR54	11-30	IR54R	586	74	12.6 (ii)

(i) Accessions classified as "*O. perennis*" have not yet been reclassified by IRGC. These accessions may be *O. rufipogon*, *O. nivara* or hybrids of either species with *O. sativa*.

(ii) Embryo rescue was necessary to ensure survival of the backcross plants.

Table 4. Chromosomal location and linkage values between linked isozyme loci.

Chromosome	Loci located and gene order with linkage values where known[a]
1	Icd-1, Got -1, Est-5
2	Amp-1
3	Amp-3/Est-2 —14— Pgi-2 —39— Pox-5 —22— Cat-1
4	Pgi-1
6	Sdh-1 —13— Pox-2 —28— Acp-1/Acp-2
7	Est-9
8	Amp-2 ---------- Amp-4
11	Adh-1 —19— Pgd-1

[a] _____ linked ---------- independent