65970

CIAT
Centro Internacional de Agricultura Tropical

APARTADO AEREO 67-13
CABLES: CINATROP
CALI - COLOMBIA

CENTRO INTERNACIONAL DE AGRICULTURA TROPICAL

TO:

Pulver Edward

Use of anther culture in rice breeding

Paper Presented at Annual Meeting of

The Rockefeller Foundation Program

on the Genetic Engineering of Rice

Held at IRRI on October 14-16, 1986

USE OF ANTHER CULTURE IN RICE BREEDING [1/]

Edward Pulver [2/]

I. INTRODUCTION

The production of doubled haploid rice plants through the culturing of anthers is a well established laboratory technique that has numerous uses in rice breeding. The most immediate and perhaps significant application of this method is as a practical breeding tool since homozygosity can be obtained in less than one year after culturing anthers from an F_1 plant.

The advantages of using the anther culture method for breeding purposes have been recognized for several years; however, a tremendous gap still exists between potential and practice. Some success as measured by the release and acceptance of new varieties has been obtained by several Chinese institutes working with japonica types with known androgenetic capabilities. However, in spite of these notable achievements, anther culture still remains to be incorporated into established rice improvement programs as a routine breeding method.

1/ Work partially financed by The Rockefeller Foundation.

2/ Rice Program, CIAT, A. A. 6713, Cali, Colombia.

Several factors have impeded the use of anther culture for breeding purposes. It is well recognized that most genotypes are not capable of producing callus tissue from microspores with subsequent regeneration of doubled haploid plantlets. As a result, most research in anther culture has been restricted to a narrow range of germplasm selected primarily for androgenetic properties rather than for their usefulness as parents in a breeding program. Genetic material that is of interest to the rice breeder has been largely neglected. Thus, anther culture is still viewed by most plant breeders as a laboratory curiosity.

Another restricting factor is the lack of suitable methods to accommodate the large volume of material essential for a breeding program. Most rice breeders are unfamiliar with anther culture, although it is understood by tissue culturalists who are not sufficiently acquainted with rice breeding to develop the required methods. Thus, success in developing anther culture as a breeding method depends largely on overcoming these limitations.

Anther culture research at CIAT (Centro Internacional de Agricultura Tropical) commenced after a considerable amount of information was available, thus enabling the work to be organized on an applied basis. The division between laboratory research and field breeding was avoided by assigning responsibility for anther culture to the rice improvement program. Work is conducted in collaboration with tissue culturalists and physical facilities are shared but the direction of anther culture research and day-to-day activities are the responsibilities of the rice improvement staff. This has resulted in an

increased awareness by the breeders of the usefulness of anther culture while anther culture activities are directed from a rice breeding perspective.

Research is restricted to genetic material employed in the breeding program resulting, initially, in low rates of callus induction and plantlet regeneration. Work has concentrated on indica rices although their ability to undergo androgenesis is much less than that of japonicas. However, much progress has been made and the doubled haploids produced using this approach have immediate commercial use.

II. OBJECTIVES OF ANTHER CULTURE RESEARCH

The rice program at CIAT has regional responsibility for assisting national institutes in improving rice production in the Americas. Due to the tropical location of the breeding program, our ability to serve temperate areas is restricted. However, there are severe needs for increased rice production in the temperate areas of Brazil, Argentina, Uruguay, Paraguay and Chile and during the cold season in Cuba. Breeding programs in these areas are limited to one generation in the field per year and as a result progress is slow; requiring approximately 15 years to produce a variety. In most of the rice area in the Southern Cone, tall semi-improved varieties are still cultivated i.e. "Bluebelle" in southern Brazil, Uruguay, Paraguay and "Fortuna" in Argentina. In Chile, European varieties introduced more than 50 years ago are still grown even through they are of poor quality and their market value is less than 50% of good quality rice.

Cuba is also a targeted area for anther culture. The country has a yearly production of approximately 500,000 tons of paddy rice but imports another 300,000 tons of milled rice annually. Varieties produced at CIAT are unadapted and cold-tolerant germplasm obtained from Asia has unacceptable grain quality. Attempts to combine the cold tolerance of Asian japonicas with the indica grain quality have not been successful. Also, Cuba has a serious hoja blanca virus (HBV) problem. To incorporate these three characters plus blast resistance into a single genotype would require 12-15 years using conventional breeding methods.

Anther culture breeding can be of tremendous value in temperate areas. The creation of homozygous lines in 7 to 8 months would reduce the time required to produce a variety from 15 years to only 5 and the potential impact of a successful program would be substantial. In Chile, the identification of cold tolerant material with good grain properties would have a commercial value of at least US$10 million annually. In southern Brazil approximately 200,000 has can be incorporated into irrigated production if suitable cold tolerant varieties can be developed. This new production could eventually contribute up to 1 million additional tons of rice, essentially equal to the amount that Brazil is forecasted to import this year. The market value of this potential harvest would exceed US$300 million annually. Appropriate varieties for Cuba could almost eliminate the need for importation resulting in an annual savings of approximately US $100 million.

Breeding via anther culture could also be useful in our acid soil, upland program, essentially restricted to one growing season per year, by significantly accelerating the development of improved material. Furthermore, the upland program is employing new sources of disease resistant material which eventually will be useful in the tropical irrigated program. The identification of new dwarf plant types from the upland program for use in the irrigated sector would broaden the genetic base of our currently narrow irrigated germplasm which is exclusively of Asian origin.

During the past year, activities concentrated on anther culture

methods suitable for use in a breeding program, identification of agronomically useful material that responds to anther culture, and processing crosses that have resulted in the production of several thousand doubled haploid plants now being tested for their agronomic usefulness in areas of potential use.

III. STUDIES ON METHODS

a. Plating of Anthers

The most laborious step in the anther culture process is the plating of anthers on the induction medium. The most commonly employed method consists of tapping cut florets on the perimeter of the induction flask thus permitting anthers to fall on the induction medium. With this technique one technician can plate approximately 1,500 anthers/day. This efficiency is acceptable when the planting of the F_1 plants being processed can be staggered. However, the exposure of the F_1 to selection pressures results in a volume of material that can exceed the capacity to plate anthers. At CIAT, all F_1 triple crosses designed for tropical America are evaluated for HBV resistance. The evaluation is conducted only twice per year since rearing sufficient vectors (Sogatodes oryzicola) of HBV is difficult. Consequently, we often have up to 400 F_1 crosses available for anther culture processing during approximately 2-3 weeks. Current methods only allow for a small percentage of the material to be processed by anther culture. Similarly, we observe that R_2 lines derived from F_2 plants previously evaluated for agronomic characters and disease resistance are much superior to doubled haploids regenerated from unscreened F_1 material. However, the volume of F_2 populations available far surpasses the ability to plate the anthers.

Two methods that could significantly reduce the effort required to extract the anthers were studied. One procedure

consists of placing cut florets directly into the induction medium and with agitation approximately 2-3 anthers can be separated from the glumes with ease. Although this procedure is less time consuming than the standard practice, bacterial contamination is a serious problem. A similar method, originally reported by Japanese scientists, consists of placing the cut florets directly into the liquid induction medium and with mild shaking during the induction process the calli are separated from the glumes. However, contamination is high often exceeding 50% of the samples. With either of the two methods surface sterilization of the glumes to reduce contamination has been only partially effective with both methods. Our evidence indicates that the source of the contaminant may be inside the florets. Current studies involve the use of strong surface sterilants combined with selected antibiotics incorporated into the induction medium. Preliminary results with this procedure indicate that contamination can be reduced to an acceptable level but the effect of the sterilants and antibiotics on androgenesis on a range of genotypes is unknown.

The ability to store harvested panicles prior to plating would also facilitate the processing of large volumes of material that is available in a short time. However, bacterial contamination is also a serious problem with stored samples, especially when the spikelets are left intact in the boot. We are currently examining various methods of treating panicles prior to storage.

Contamination is seldom mentioned as a constraint in anther

culture work; however, we have experienced serious problems. Some of the contamination could be due to techniques, but a better explanation may be the treatment of the source of the anthers prior to collection. We extract anthers mostly from material after disease exposure especially HBV and Piricularia oryzae. In contrast, most investigators report the use of greenhouse-grown plants, or, if field grown, in the absence of disease pressure. However, the benefits gained from culturing anthers only from screened material far outweigh the reduced contamination resulting from growing unexposed plants. Consequently, our efforts are directed toward reducing contamination in preference to culturing material that comes from a clean environment. At present, we are limited to working with freshly harvested material and germplasm that has a rather high level of callus induction ability.

More research is required on methods of plating anthers to allow the processing of more material, including germplasm that possesses only a low level of androgenesis.

b. Induction Media

The most studied area of rice anther culture is the influence of induction media on callus formation. Unfortunately, most of the work has been conducted using a narrow japonica germplasm base and similar efforts have not been devoted to indica types. The induction medium (including various hormones) is important but it is not the controlling factor for callus formation. Induction media have quantitative influences on callus induction but the

media are not capable of converting a non-responsive genotype into a highly responsive one.

Initial results at CIAT suggested a significant genotype X induction medium interaction. However, more detailed studies did not support these earlier observations. Results using anthers extracted from 24 hybrids of diverse crosses clearly demonstrated that the potato extract medium containing 4 ppm of naphthaleneacetic acid (NAA) and 1 ppm kinetin was superior to the same basal medium but with 2,4-D as the auxin or the N_6 medium containing NAA and kinetin (Table 1). The interaction of genotype X medium was not significant. In 22 out of 24 hybrids the potato extract medium with NAA resulted in more calli than the other two media and in the remaining 2 hybrids it was equally as good as the same basal medium with 2,4-D added (Fig. 1). Consequently, we employ the potato extract medium with 4 ppm NAA and 1 ppm kinetin throughout. New media are being evaluated but it is not anticipated that a vastly superior induction medium will be found.

c. Influence of Temperature Pre-treatment

The use of a cold shock to stimulate androgenesis of japonica types is well documented; however, we question this method for tropical indicas having pollen sensitive to low temperatures. Anthers from three tropical indica varieties and one japonica were subjected to various temperature pre-treatments and incubated at 25°, 30° and 35°C. The results show that callus induction of the tropical variety "CICA 8" is increased by pre-treating the anthers

at 8°C for 15 days (Table 2). "Fanny" (japonica type) required only a 10 day treatment and 15 days of cold shock was similar to the un-pretreated check. Pre-treating anthers at 30°C for only 5 days drastically reduced androgenesis in all varieties. Short exposures at 35°C or 40°C either had a negative effect or no influence on the indica types, but significantly reduced callus formation of Fanny. The incubation temperature is also important as temperatures above 25°C completely inhibited callus induction in both the indicas and Fanny.

The influence of low temperature pre-treatment on callus induction appears to be similar to the effect of the induction medium; that is, it is quantitative. The use of cold shock can stimulate induction in material that possesses androgenetic properties but it will not significantly alter induction of a low calli-producing genotype.

d. Influence of Regeneration Medium

The regenerative culture medium appears to have less influence on plant regeneration than the carry-over effects of the induction medium. The commonly used media for regeneration are standard media used for calli from rice and other various crops as well as vegetative tissues. The solidified Murashige-Shoog (MS) medium containing 1 ppm NAA + 4 ppm kinetin is our standard and we have not encountered media that are superior.

The regeneration environment, both chemical and physical,

plays an important role in regeneration. Genetic factors are obviously more critical but tremendous variation for plant regeneration is frequently observed even from calli derived from genetically pure material. The source of this variation is unknown, but normally 80% of the calli that are capable of undergoing plant regeneration will exhibit a green spot within 10 days after transfer to the regeneration medium. This suggests that the development of the callus at the time of transfer is critical. Calli exposed to the induction medium for long periods seldom regenerate plants. Consequently, early transfer of calli to the regeneration medium increases the probability of regeneration. However, very small calli die if transferred too early. Methods that assure survival of tiny calli would enable the transfer of minute tissue and thus increase plant regeneration. Commercial grade agar results in high mortality of small calli whereas purer agar, as well as cotton, dramatically increase callus survival and the percent of plant regeneration (Table 3). Regeneration is sensitive to various types of contaminants (metals, toxins, etc) and care should be taken in media preparation.

At present we are using the MS medium with 1 ppm NAA + 4 ppm kinetin semi-solidified with Gel-rite. Cotton support is equally good but difficulties are encountered in removing regenerated plants. Commercial agar is not a satisfactory support medium for regenerating plants from anther-derived callus tissue.

e. Uniformity of Regenerated Plants

Individual calli are products of single microspores and are easily maintained separately on liquid induction media. However, some researchers e. g. IRRI and the Shanghi Academy separate the clusters of plantlets originating from a single callus into individual plantlets, even through these plantlets should be genetically identical. Although this procedure increases the number of regenerated plants, the genetic diversity remains constant. The increased number of regenerated plants, many of which may be clones, complicates the evaluation of R_2 lines, especially for multiple factors i. e. grain quality, iron toxicity, cold tolerance, and disease resistance.

We observe that plantlets derived from an individual callus are phenotypically equal. Callus dissected in the early "green spot" stage to produce several green calli regenerate identical plants. Also, seeds harvested in bulk from unseparated plants originating from one callus do not produce significant amounts of segregating R_2 lines. Less than 5% of the R_2 lines demonstrate off-types which are discarded at harvesting and phenotypically identical plants bulked. Consequently, all regenerated plants discussed here are from individual calli and exclude plantlets from the same callus.

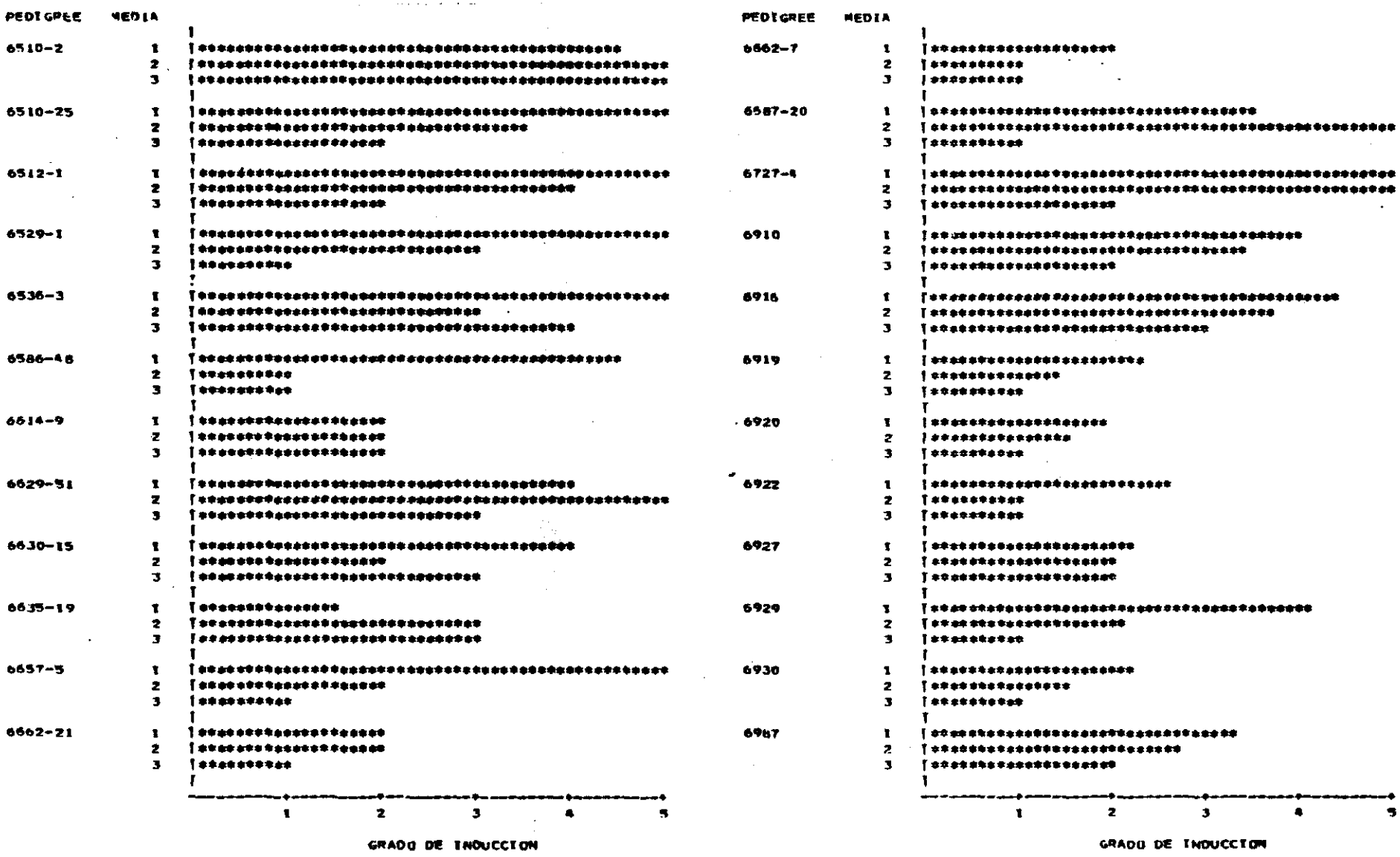

Fig. 1. Rate of androgenesis of pollen from 24 hybrids cultured in three induction media. Callus induction estimated using scale of 1-5: 1= no callus; 2 = < 10; 3 = 10-25; 4 = 25-50; and 5 = > 50 calli/ 100 anthers.

Table 1. Effect of induction medium on callus formation.

Induction Medium	Rate of Callus Formation [1]
Potato extract + 4 ppm NAA + 1 ppm Kinetin	3.01 ± .08 [2]
Potato extract + 2 ppm 2,4-D + 1 ppm Kinetin	2.56 ± .11
N 6 + 4 ppm NAA + 1 ppm Kinetin	1.79 ± .10

[1] Scale 1 - 5: 1 = no callus; 2 = < 10; 3 = 10-25; 4 = 25-50; and 5 = > 50 calli/100 anthers.

[2] Values are means of 24 F_1 hybrids with a total of 162 observations followed by standard error.

Table 2. Influence of temperature pre-treatment of anthers on callus formation of four rice varieties [1/]

Pre-treatment	Incubation Temp.	Variety [2/] CICA 8	CICA 4	Oryzica 1	Fanny	Treatment mean [3/]
		No. of Calli/100 anthers				
None	25°C	8.0	3.3	0.6	338	87.5
8°C 5 days	25°C	10.8	0.7	0.4	415	106.7
8°C 10 days	25°C	12.2	0.3	2.0	504	129.6
8°C 15 days	25°C	27.5	0	0	300	81.9
None	30°C	0	0	0	11	2.6
30°C 5 days	25°C	0	0.8	0	1	0.5
30°C 10 days	25°C	0	0	0.4	4	1.1
30°C 15 days	25°C	0	0.2	0	1	0.3
None	35°C	0	0	0	8	1.9
35°C 1 day	25°C	2.2	1.6	2.2	83	22.3
35°C 2 days	25°C	13.8	0.3	0.6	20	8.7
35°C 4 days	25°C	1.0	6.0	0.4	62	17.3
40°C 6 hours	25°C	6.8	0	0	131	34.5
40°C 12 hours	25°C	10.0	1.8	0	83	23.6
40°C 24 hours	25°C	2.6	0.2	0	98	25.2

1/ After pre-treatment anthers were incubated at 25°C for 50 days.

2/ LSD(.05) for comparisons of treatments within CICA-8 = 5.5; within CICA-4 = 1.4; within Oryzica-1 = 1.0; and within Fanny = 103.

3/ LSD(.05) for comparisons of treatment means = 14.9.

Table 3. Effect of support media on callus survival and green plant regeneration.

	Callus Mortality				Plant Regeneration			
	Support Media 1/							
Variety	Ar	COT	Ag	G-R	Ar	COT	Ag	G-R
	(%)							
TOX 1010-49-1	70 ± 8.6	58 ± 3.9	51 ± 4.6	59 ± 3.8	4.6 ± 1.4	6.6 ± 1.3	7.4 ± 2.2	4.9 ± 1.6
IAC 165	65 ± 6.3	54 ± 2.5	41 ± 4.3	55 ± 6.2	1.0 ± 0.9	2.0 ± 1.3	3.0 ± 3.5	5.0 ± 2.2
TOX 1011-4-1	63 ± 10	66 ± 9.8	53 ± 6.4	54 ± 9.5	4.3 ± 2.9	5.0 ± 3.3	11.4 ± 3.4	5.7 ± 3.7
Colombia 1	68 ± 4.8	38 ± 7.0	63 ± 5.6	48 ± 12	0	12.5 ± 1.3	10.0 ± 5.1	13.3 ± 5.6
Col.1XM312A	97 ± 3.3	40 ± 5.8	58 ± 8.6	50 ± 11	0	0	2.5 ± 2.0	0
Treatment means 2/ :	70 ± 7.7	55 ± 4.8	51 ± 5.1	56 ± 6.1	3.3 ± 1.3	5.8 ± 1.5	7.2 ± 2.5	5.5 ± 2.2

1/ Support media: Ar = Commercial grade Agar; COT = Cotton; Ag = Agarosa; G-R = Gel-rite.

2/ Values are means of 64 replications with 10 calli/rep. followed by standard error.

IV. Development of Cold Tolerant Germplasm

a. Identification of Parents

National programs in Cuba, Chile and southern Brazil as well as CIAT have attempted for many years to combine the cold tolerance of some japonicas with indicas that possess good grain quality, but these efforts have been unsuccessful. Incompatibility between japonicas and indicas restricts conventional breeding efforts in this area. However, calli can be induced from pollen of crosses that are highly sterile (Table 4), permitting the anther culture of crosses involving cold-tolerant japonicas with good quality indicas.

The ability to induce callus in several known cold tolerant genotypes is shown in Table 5. The Chilean japonica varieties have excellent callus induction, often exceeding 900 calli/100 anthers. This is our most responsive material in terms of callus formation. Based upon their known cold tolerant properties and their androgenetic ability these genotypes were selected as parents.

b. Regeneration of Doubled Haploids

The principal defect of the Chilean germplasm is unacceptable grain cooking quality. Consequently, the first set of crosses was designed to combine the excellent grain quality of "Lemont" (US variety) that has modest cold tolerance with the highly cold tolerant Chilean germplasm. Three-way crosses were made combining two cold tolerant chilean sources with Lemont.

Material was processed via anther culture using techniques previously described. A summary of the anther culture process is presented in Table 6. An arbitrary goal of approximately 150 doubled haploids/cross was set. However, some crosses were agronomically superior to others (i. e. CT 6741); consequently, more anthers were plated from these crosses. In general, the crosses were easily processed via anther culture due to their high androgenetic ability. Callus induction as measured by the number of anthers plated was approximately 37%, and almost 9% of the calli were capable of undergoing plant regeneration. Further, approximately 3/4 of the green plants spontaneously doubled and produced doubled haploids.

The same crosses were also processed using conventional methods to compare the performance of lines derived from anther culture with those from conventional breeding methods. Heavy sterility was observed and under normal conditions these crosses would have been discarded. Only 234 F_2 families were formed from 10 crosses. A comparison of the conventional breeding method with anther culture is presented in Fig. 2.

High selection pressure was made at CIAT for earliness, plant type and grain quality resulting in the identification of 190 R_3 lines combining earliness, semi-dwarfism, long grain, clear endosperm, intermediate gelatinization temperature and an amylose content of 21-25%. Similarly, approximately 270 F_5 lines were derived using the conventional approach. Due to the low number of

F_2 families formed using the conventional method, the 190 R_3 lines from anther culture may be more genetically diverse. The main difference between the methods is time; the 190 R_3 lines were regenerated in 7 months whereas the F_5 lines required four generations. This material is presently being evaluated for cold tolerance in Chile and southern Brazil. However, considering the parentage of the crosses we are confident that several of these selections will exhibit excellent cold tolerance.

Cold tolerant material with good grain and plant types is now available. To our knowledge, this is the first time that such material has been produced. This germplasm has tremendous immediate potential for Chile and other temperate areas such as USA, and southern Europe and will serve as donors for cold tolerance in Cuba and southern Brazil for combination with HBV and blast resistance and iron toxicity tolerance. These new crosses are in progress.

c. Southern Brazil

The extreme south of Rio Grande do Sul requires varieties that possess cold and iron toxicity tolerance, blast resistance, and earliness. Approximately 100 triple crosses were made involving these combinations. Chilean germplasm was used as the cold tolerant donors and material from our tropical breeding program as sources for iron tolerance and blast resistance. Unfortunately, these wide crosses resulted in almost complete sterility and less than 20 crosses were processed using the conventional method. Anther

culture was not much superior as 60 of the 98 crosses failed to produce calli. This is not surprising as irrigated germplasm from the tropical breeding program has a very low level of androgenesis. Of the 38 crosses subjected to anther culture, 758 doubled haploids were regenerated but only 92 possess good grain quality combined with earliness and acceptable plant type (Fig. 3). These lines are presently being evaluated for iron tolerance and blast resistance as well as adaptation to the conditions in southern Brazil.

Table 4. Relationship between callus induction ability and sterility of different F_1 plants from four triple crosses involving japonica and indica types.

Cross: Diamante/IRGA 410//P2015-F_4-66		Cross: Lemont/SI-2//Diamante	
Callus Induction	Sterility	Callus Induction	Sterility
%		%	
29	98	30	98
26	98	27	98
9	98	94	95
9	98	58	95
138	95	20	95
41	95	66	90
14	95	100	85
1	95	47	85
39	15	68	60
28	15	84	50

Cross: Lemont/Diamante//P2015-F_4-66		Cross: Lemont/IR19743-25-2//Diamante	
Callus Induction	Sterility	Callus Induction	Sterility
%		%	
13	98	31	98
6	98	2	98
10	95	32	95
100	80	2	95
0	80	14	90
78	70	7	80
1	70	6	70
80	40	3	50
16	30	0	50
3	30	0	15

Table 5. Callus formation ability of cold tolerant material selected from Asian nurseries and cold tolerant Chilean germplasm.

Entry	Rate of Callus Formation 1/
I 32	1.3
SR 4079-4-2	1.0
Zho Fee No. 10	1.0
China 988	1.0
China 1039	1.0
IR 9202-5-2-2-2	1.0
K 31-163-3	1.0
K 39-96-1-1-1-2	1.0
Tainung sin yu	1.0
Stejaree 45	5.0
Material from Chile	
Diamante	5.0
Q - 65101	5.0
Q - 64117	5.0
Q - 66304	5.0
Q - 67103	5.0

1/ Scale 1 - 5: 1 = no callus; 2 = < 10; 3 = 10-25; 4 = 25-50; and 5 = > 50 calli/100 anthers.

Table 6. Efficiency of 10 triple crosses designed for Chile in the production of doubled haploids via anther culture.

Pedigree	Cross	N° of Anthers Plated	N° of Calli Produced	% Induction	Plants Regenerated			Green Plant Production			Efficiency in Renegerating Doubled Haploids		
					Total	Green	Albinos	% Calli	% Anthers	N° Doubled Haploids	% Green Plants	% Calli	% Anthers
CT 6741	Diamante/Lemont//Q 65101	11000	4436	40.3	602	311	291	7.0	2.8	211	67.8	4.8	1.9
CT 6742	Q 64117/Lemont//Q 65101	2900	1944	67.0	284	108	176	5.5	3.7	69	63.9	3.5	2.4
CT 6743	Q 65101/Lemont//Q 65101	3000	491	16.3	94	53	41	10.7	1.7	28	52.8	5.7	0.9
CT 6744	Q 66304/Lemont//Q 65101	3700	2001	54.1	218	96	122	4.7	2.6	79	82.3	3.9	2.1
CT 6745	Q 67103/Lemont//Q 65101	1700	464	27.3	34	43	34	9.2	2.5	22	51.2	4.7	1.3
CT 6746	Diamante/Lemont//Diamante	3100	1641	52.9	348	245	103	14.9	7.9	169	69.0	10.3	5.5
CT 6747	Q 64117/Lemont//Diamante	2900	753	26.0	115	67	48	8.9	2.3	50	74.6	6.6	1.7
CT 6748	Q 65101/Lemont//Diamante	2500	1109	44.3	236	179	57	16.1	7.2	145	81.0	13.1	5.8
CT 6749	Q 66304/Lemont//Diamante	7100	1046	14.7	184	123	60	11.7	1.7	116	94.3	11.1	1.6
CT 6750	Q 67103/Lemont//Diamante	1200	496	41.3	87	56	31	11.3	4.7	52	92.9	10.5	4.3
Summary		39,100	14,381	36.8	2,202	1,281	963	8.9	3.3	941	73.5	6.5	2.4

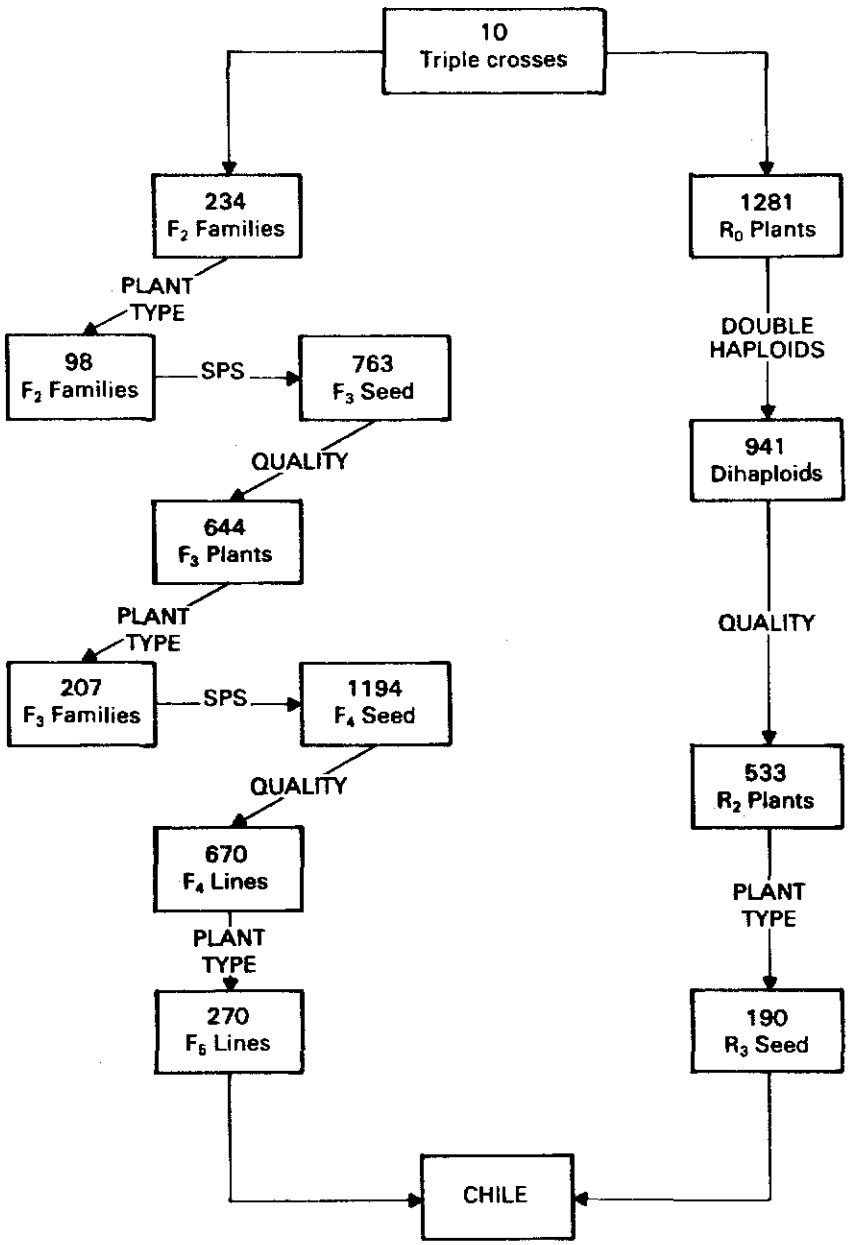

Fig. 2. Flow of germplasm from crosses designed to combine cold tolerance with indica grain quality employing conventional breeding methods and anther culture.

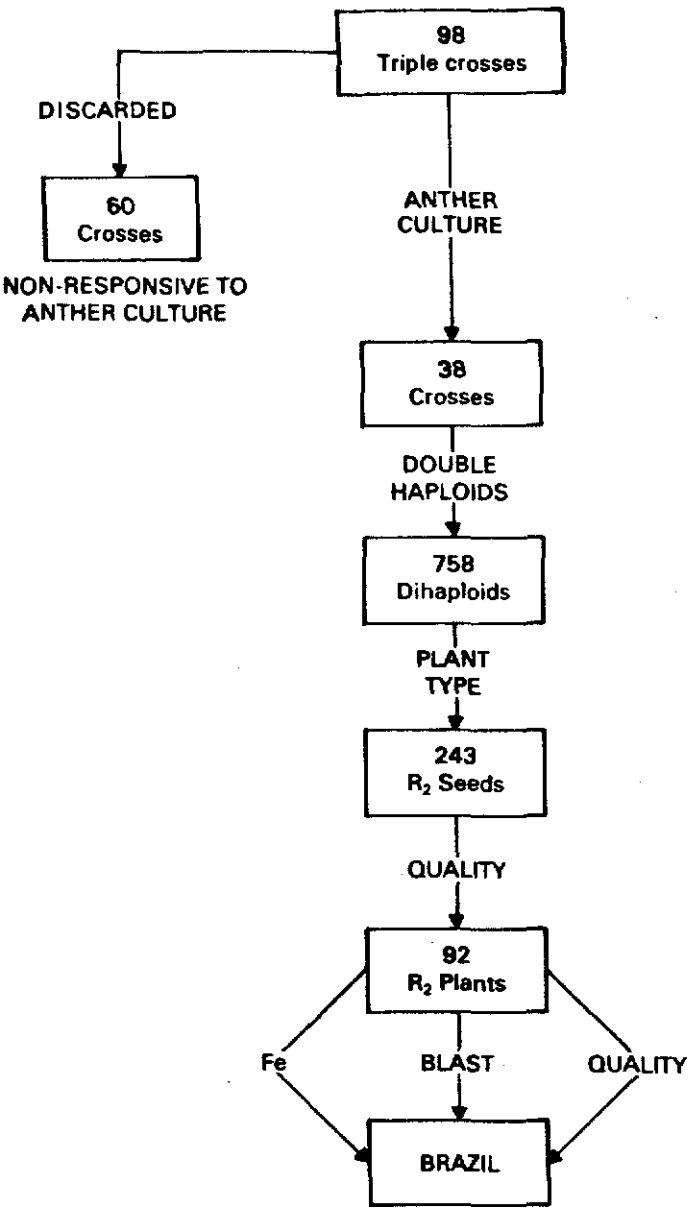

Fig. 3. Diagram of crosses programed for southern Brazil and processed using anther culture methodology.

V. ADVANCES IN THE ACID SOIL UPLAND BREEDING PROGRAM

a. Germplasm Evaluation

The callus forming ability of some commonly used parents from the upland breeding program (acid soil ecology) is generally greater than that of parents from the irrigated program (Table 7). Several genotypes from the upland program possess the ability to form callus tissue which is not the case in the irrigated germplasm. Based upon this and other parental evaluations, several crosses from the upland program were processed via anther culture.

The objectives of the upland program are to combine tolerance to soil acidity with intermediate plant type and resistance to HBV and other tropical diseases. Resistance to HBV is controlled by a single dominant gene; consequently, the F_1 of triple crosses having one resistant parent is evaluated for resistance. This early evaluation allows for removal of the homozygous susceptibles, and anthers were taken only from the heterozygous plants after HBV screening. Theoretically, 50% of the doubled haploids regenerated using this procedure are resistant to HBV.

A frequency distribution for callus induction and plant regeneration of 68 F_1 triple crosses is shown in Fig. 4. More than 50% of the crosses exhibited sufficient callus formation for processing via anther culture. Furthermore, about one-half of the crosses that possess androgenetic properties produced calli that resulted in greater than 10% plant regeneration. Crosses that exhibited acceptable levels of callus formation and plant

regeneration are listed in Table 8. Two commonly occurring parents in the better crosses are TOX 1871-38-1 and TOX 891-212-2-102-2-101 both of which possess high levels of callus induction and plant regeneration (Table 7).

From these 68 crosses approximately 22,000 anthers were plated resulting in 8,000 calli. While only 5% of the calli regenerated green plants, 69% of these were doubled haploids. Albinism was extremely high and appears to be common in crosses from the acid soil program.

The same procedure was repeated following the 1986 first season hoja blanca screening. Anthers were removed from Rr plants after virus screening in crosses that exhibited a high proportion of semi-dwarf plant types. The callus forming ability of these crosses is presented in Table 9. More than 50% of the crosses were capable of forming sufficient calli to facilitate processing via anther culture and 15 crosses exhibited exceptional plantlet regeneration. Over 16,000 calli were obtained resulting in approximately 1,000 R_1 plants. We expect that about 600 these will be doubled haploids.

Anthers have also been collected from F_2 populations grown under extremely high blast pressure in the Colombian Llanos. This material was difficult to process due to high levels of bacterial contamination. However, 143 doubled haploids were regenerated and most of these exhibit excellent plant type and good disease

resistance. Although a generation is lost by processing the F_2 generation, the superior doubled haploids produced justify this procedure.

Advanced breeding lines from the upland program have also been evaluated for androgenesis. Of 16 F_4 lines that possess semi-dwarf plant types combined with HBV and disease resistance four exhibit sufficient callus induction and regeneration to be considered as parents for use in anther culture (Table 10). These lines will be crossed with selections from the cold-tolerant Chilean anther culture-derived material and doubled haploids will be regenerated for testing in Cuba.

In summary, more than 1,000 doubled haploids have been regenerated from the upland breeding program (Table 11). These crosses were made independently of anther culture considerations, yet more than 35% of the anthers collected from a range of material produced calli. This clearly illustrates the high callus induction capability present in the upland germplasm which is largely African in origin. However, only approximately 5% of the calli regenerate green plants. A comparison between the anther culture efficiency of the Chilean and the upland material shows the higher rate of albinism in the acid soil crosses (Table 12).

b. Perspectives

The doubled haploids from the upland program represent germplasm that has tremendous potential. Many are semi-dwarfs

under irrigated conditions but maintain the deep-rooted character typical of upland material. Approximately 30% of the R_2 lines tested are also highly resistant to blast and 50% should be resistant to HBV. Furthermore, many of the lines are early, maturing in less than 110 days under tropical conditions and most are highly tolerant to iron toxicity. This new germplasm should be extremely useful in the irrigated program as it presents sources of disease resistance and other characters (e.g. earliness) completely distinct from the currently available irrigated germplasm.

Lines derived from the upland program will be crossed with the Chilean germplasm to combine cold tolerance with blast resistance and Fe toxicity tolerance to meet the conditions in southern Brazil. Also, the R_2 lines with HBV and blast resistance will be combined with the Chilean material to fulfill the requirements for Cuba. These crosses will be processed using anther culture as all parents have known androgenetic abilities.

Table 7. Callus forming ability of anthers isolated from parents that are frequently used in the upland and irrigated breeding programs.

Upland Program	Rate of Callus Formation [1/]	Irrigated Program	Rate of Callus Formation [1/]
COL 1 X M312A	2.1	IR 25840-64-1-3	1.0
COL 1	2.1	GZ 864-2-3-1	4.0
IRAT 122	1.9	ECIA 24-107-1	1.0
IRAT 124	1.2	P 2867 F_4-52-2	1.0
TOX 1010-49-1	4.3	P 2887 F_4-9-4	1.2
TOX 1011-4-1	2.3	P 3059 F_4-25-3	1.6
TOX 1737-103-4	4.0	P 3299 F_4-86	1.0
TOX 1768-1-2-1	1.8	16404	1.4
TOX 1768-1-2-2	2.0	17361	1.0
TOX 1780-2-1-1P-2	2.0	18521	1.0
TOX 1785-19-18	4.5	23916	1.0
TOX 1837-103-4	1.0	26444	1.0
TOX 1859-102-4M-4	1.3	11219	1.0
TOX 1871-38-1	4.5		

[1/] Scale 1 - 5: 1 = no callus; 2 = < 10; 3 = 10-25; 4 = 25-50; and 5 = > 50 calli/100 anthers.

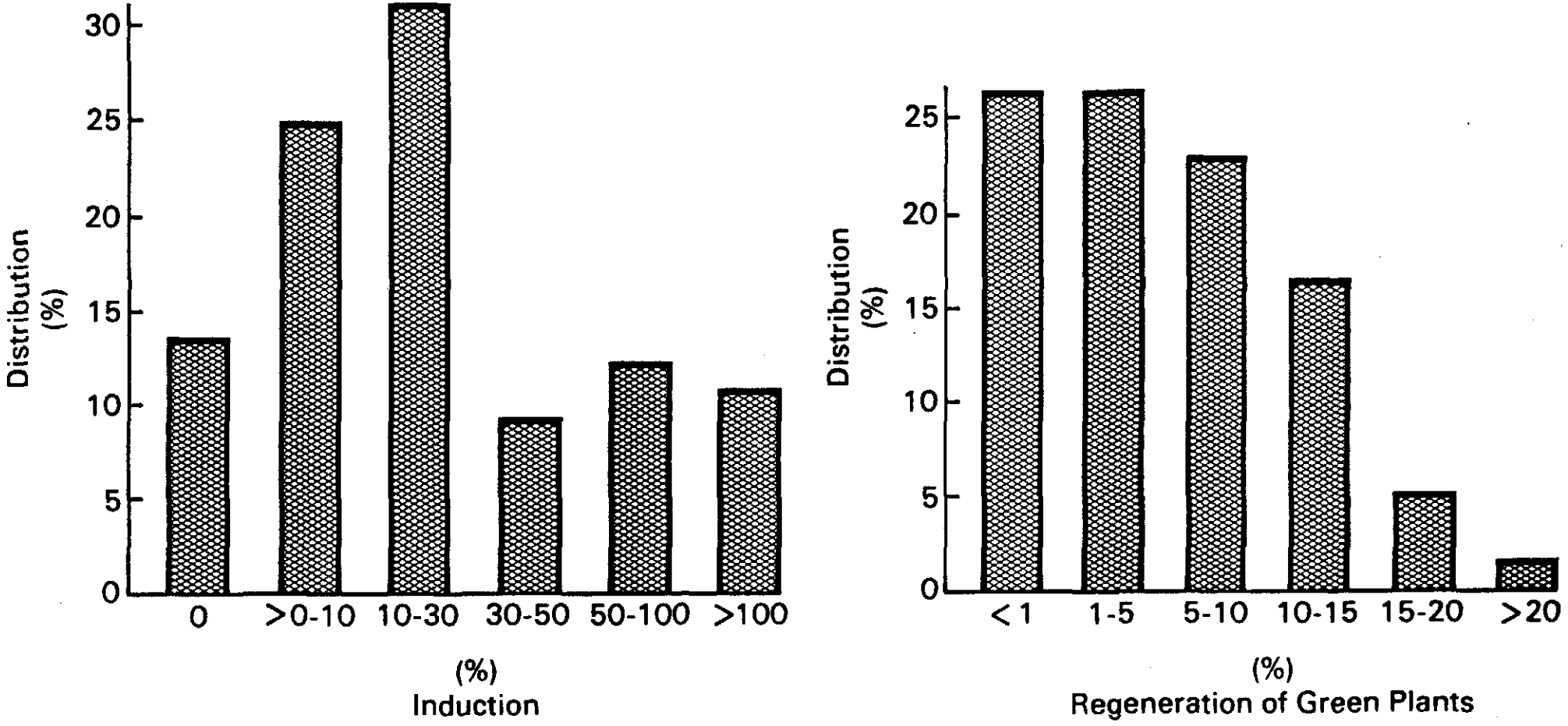

Fig. 4. Frequency distribution for the ability to undergo callus induction and plant regeneration of 68 F_1 hybrids from the acid soil, upland ecosystem.

Table 8. F_1 hybrids from crosses made for the acid soil, upland ecosystem that have acceptable levels of callus formation and plant regeneration.

Pedigree	Cross	% Induction	% Regeneration
CT 7488	IRAT 194-1-2-B/IRAT 122//TOX 1780-2-3-2-1-2	30	14
CT 7422	NGOVIE/COL 1 X M312A-74-2-8-8//TOX 891-212-2-102-2-101	25	11
CT 7415	TOX 939-107-2-101-1-1B/COL 1 X M312A//TOX 1780-2-1-1P-4	23	12
CT 7413	TOX 1780-2-3-201-1/COL 1 X M312A//IAC 165	23	13
CT 7395	BR 51-282-8/COL 1 X M312A//TOX 891-212-2-102-2-101	23	16
CT 7484	ITA 133/COL 1//TOX 891-212-2-102-2-101	17	15
CT 7500	TOX 1010-49-1/IRAT 122//TOX 1871-38-1	16	15
CT 7411	TOX 906-2-1-202-2/COL 1 X M312A//TOX 1871-38-1	15	17
CT 7469	ITA 133/COL 1 X M312A-74-2-8-8//TOX 891-212-2-102-2-101	14	11
CT 7428	TOX 1010-22-7-16/COL 1 X M312A//TOX 891-212-2-102-2-101	12	19

Table 9. Callus induction ability of 48 F_1 triple crosses from the upland breeding program after screening for resistance to the hoja blanca virus.

Cross	Rate of Callus Formation 1/	Cross	Rate of Callus Formation 1/
CT 7641	1.0	CT 7607	3.7
CT 7650	1.4	CT 7637	4.0
CT 8077	1.4	CT 8081	4.1
CT 7765	1.7	CT 7743	4.1
CT 8058	1.8	CT 7619	4.2
CT 8091	1.9	CT 7747	4.2
CT 7649	2.0	CT 7721	4.3
CT 7717	2.0	CT 7818	4.3
CT 7638	2.0	CT 7621	4.3
CT 7628	2.1	CT 7614	4.4
CT 8105	2.1	CT 7718	4.5
CT 7646	2.3	CT 8060	4.5
CT 7734	2.3	CT 7808	4.6
CT 8076	2.3	CT 7634	4.6
CT 7742	2.4	CT 8071	4.7
CT 7606	2.4	CT 7813	4.7
CT 7625	2.5	CT 7629	4.7
CT 8070	2.7	CT 7639	4.9
CT 7615	2.8	CT 7832	4.9
CT 7652	3.0	CT 8065	4.9
CT 7744	3.0	CT 7746	5.0
CT 8085	3.0	CT 7622	5.0
CT 7656	3.1	CT 7633	5.0
CT 7642	3.2	CT 7833	5.0

1/ Scale 1 - 5: 1 = no callus; 2 = < 10; 3 = 10-25; 4 = 25-50; and 5 = > 50 calli/100 anthers.

Table 10. Response to anther culture of advanced dwarf, upland breeding lines that are resistant to hoja blanca virus and tropical fungal diseases, tolerant to iron toxicity and mechanical damage from Sogatodes oryzicola.

Pedigree	Rate of Callus Formation [1]/	% of Calli Regenerating Green Plants
CT 6424-12-1-1	5.0	0.7
CT 6516-21-8-1	4.0	0
CT 6516-21-8-2	4.0	9.0
CT 6516-21-8-3	3.0	0
CT 6516-21-8-4	4.0	0
CT 6516-21-8-5	4.0	5.3
CT 6516-21-8-7	4.6	3.3
CT 6516-23-10-4	3.0	3.3
CT 6516-23-10-5	5.0	8.6
CT 6516-23-10-12	3.4	2.5
CT 6516-24-6-2	3.5	0
CT 6650-5-7-2	1.4	0
CT 6650-5-7-3	1.5	20 [2]/
CT 6650-5-7-7	2.0	0
CT 6687-23-3-2	2.5	0
CT 6687-23-3-3	3.0	0

[1]/ Scale 1 - 5: 1 = no callus; 2 = <10; 3 = 10-25; 4 = 25-50; and 5 = > 50 calli/100 anthers.

[2]/ Data on only 15 calli.

Table 11. Efficiency in regenerating doubled haploids from crosses for the acid soil, upland ecosystem.

Source of Pollen	No. of Anthers Plated	No. of Calli Produced	% Induction	No. of Green Plants	Regenerated Plants % Calli	No. of Doubled Haploids
F_1- CIAT-85B	21,600	8,005	37.1	401	5.0	276
F_2- Savanna-85A	41,000	13,476	30.4	198	1.5	143
F_3- CIAT-85B	25,400	7,553	29.7	346	4.5	163
F_1- CIAT-86A	39.000	16.083	75.4	957	6.0	600 1/
Total	127,000	45,117	-	1,902	-	1,182

1/ Estimated: data incomplete.

Table 12. Rate of albino and green plant regeneration and efficiency of doubled haploid production from calli derived from F_1 hybrids of crosses for the acid soil, upland ecosystem as compared to Chilean crosses.

Source of anthers	Regenerated Plants				Total	Doubled Haploids
	Albins		Green Plants			%
	No.	%	No.	%		Green Plants
F_1 - Upland-86B	3,019	76	945	24	582	61.6
F_1 - Upland-86A	13,330	93	957	7	600	62.7
F_1 - Chile	963	42	1281	58	941	73.5

VI. ACTIVITIES IN THE IRRIGATED PROGRAM

a. Germplasm Evaluation

The scarcity of genetic material that can produce callus is the principal limitation for using anther culture in the irrigated breeding program. Table 13 presents the callus formation properties for several high-yielding, irrigated varieties developed at IRRI and CIAT. None possesses sufficient callus induction ability for anther culture, nor do any of the other Latin American (Table 14), irrigated varieties. Only "IAC 47", an upland variety, responds sufficiently to use it in crosses for processing via anther culture.

These results are not surprising as nearly all Latin American material is indica developed from a narrow Asian germplasm base. If "IR 8", a widely used parent, possessed androgenetic capability then we would expect our commercial varieties to respond also. This is supported by data in Table 15. F_5 lines from atypical irrigated crosses (use of HBV resistant donors from japonica, African and upland sources) demonstrate some callus induction and three lines have a high ability to produce callus.

The large difference between the responsiveness to anther culture of the upland and the irrigated germplasm signifies that the upland germplasm is still genetically diverse or, conversely, the irrigated germplasm is extremely narrow. This serious limitation must be corrected to avoid disease epidemics. The newly produced upland dwarfs can significantly widen the genetic base of

the irrigated program and thus greatly reduce genetic vulnerability.

Table 13. Callus formation of some commercial irrigated varieties developed at IRRI and CIAT.

IRRI Material	Rate of Callus Formation [1/]
IR 5	1.0
IR 8	1.5
IR 36	1.0
IR 40	1.0
IR 43	1.0
IR 50	1.0
IR 56	1.0
IR 58	1.0
IR 60	1.0
CIAT Material	
CICA 4	1.0
CICA 6	1.0
CICA 7	1.6
CICA 8	1.2
CICA 9	1.0
Metica 1	1.0
Oryzica 1	1.4
Oryzica 2	1.0

[1/] Scale 1 - 5: 1 = no callus; 2 = < 10; 3 = 10-25; 4 = 25-50; and 5 = > 50 calli/100 anthers.

Table 14. Response to anther culture of some commercial varieties grown in Latin America.

Variety	Country where Cultivated	Rate of Callus Formation [1/]
Anayansi	Panama	1.0
Araure 2	Venezuela	1.0
Araure 3	Venezuela	1.0
Araure 4	Venezuela	1.0
Bamoa A 75	Mexico	1.0
Bluebonnet 50	Several	3.0
BR IRGA 409	Brazil, Argentina	1.0
BR IRGA 410	Brazil, Argentina	1.2
BR IRGA 411	Brazil, Argentina	1.0
Campeche A 80	Mexico	2.5
Camponi	Surinam	1.2
Ceysvoni	Surinam	2.0
CR 201	Costa Rica	1.3
CR 1113	Costa Rica	1.0
Culiacán A 80	Mexico	1.0
Damaris	Panama	1.0
Diwani	Surinam	1.0
Eloni	Surinam	1.0
EMPASC 101	Brazil	1.8
EMPASC 102	Brazil	1.0
EMPASC 103	Brazil	1.0
EMPASC 104	Brazil	1.0
IAC 1278	Brazil	1.0
IAC 47	Brazil	4.7
IAC 165	Brazil	2.5
INIAP 7	Ecuador	1.0
INIAP 415	Ecuador	1.6
INTI	Peru	1.0
Juma 58	Dominican Republic	1.0
PA 2	Peru	1.0
Sinaloa A 80	Mexico	1.0
Tikal 2	Guatemala	1.0

[1/] Scale 1 - 5: 1 = no callus; 2 = < 10; 3 = 10-25; 4 = 25-50; and 5 = > 50 calli/100 anthers.

Table 15. Response to anther culture of irrigated selections possessing tolerance to Fe toxicity, resistance to HBV, tolerance to Sogatodes oryzicola, acceptable grain and good plant types.

Pedigree	Rate of Callus Formation [1/]	Pedigree	Rate of Callus Formation [1/]
P 5387-3-1-3-1	5.0	P 5746-18-11-1-5	2.0
P 5404-32-4-1-2	1.0	P 5747-12-9-1-2	1.0
P 5404-32-4-1-10	1.0	P 5747-12-9-2-7	1.0
		P 5747-12-9-3-7	1.7
P 5413-8-3-1-1	1.0	P 5747-13-7-4-2	1.0
P 5413-8-3-1-3	1.3	P 5747-13-7-4-5	1.5
P 5413-8-3-2-2	1.0	P 5747-13-7-4-7	1.0
P 5413-8-3-2-4	1.0	P 5747-13-8-2-2	1.0
		P 5747-13-8-3-1	1.0
P 5419-2-17-2-8	2.0	P 5747-13-8-3-3	1.0
P 5419-2-17-2-9	1.0	P 5747-13-8-4-1	2.0
P 5419-2-17-6-1	1.0	P 5747-14-11-1-1	1.5
P 5419-2-17-6-3	1.0	P 5747-21-4-1-3	1.0
P 5419-2-17-6-4	1.2	P 5747-24-5-1-3	1.5
P 5419-2-20-5-3	1.8	P 5747-24-5-1-4	2.0
		P 5747-24-5-1-5	1.0
P 5690-1-4-1-1	1.0		
P 5690-1-4-4-1	2.0		
P 5690-1-4-4-2	1.5	P 5756-3-3-1-1	2.5
P 5690-1-4-4-3	1.0	P 5756-3-4-2-5	2.0
P 5690-1-4-4-4	1.0	P 5756-3-4-3-1	2.0
P 5690-1-4-4-5	1.5	P 5756-3-4-3-4	1.3
P 5690-1-4-4-6	1.0	P 5756-3-5-3-2	1.3
P 5690-1-11-1-7	1.0		
P 5690-1-11-4-1	1.0	P 5446-6-6-1-13	3.5
P 5690-3-7-2-2	1.0		
P 5690-3-7-2-7	2.0	P 5583-4-12-3-1	4.0

[1/] Scale 1 - 5: 1 = no callus; 2 = < 10; 3 = 10-25; 4 = 25-50; and 5 = > 50 calli/100 anthers.

VII. FUTURE ACTIVITIES

a. Methods for Processing Material

A major constraint in using anther culture is the inability to handle large volumes of breeding material. This is especially true for crosses that were not programmed for anther culture, due to a high probability of encountering material with low androgenesis. Furthermore, this problem is intensified when material for anther culture is simultaneously being evaluated for other characters, e. g. HBV resistance. We are working on better techniques for plating anthers to reduce this constraint.

It is more practical to pre-evaluate F_1 crosses for callus formation and then submit only selected crosses to anther culture. Thus, anther culture complements the conventional breeding program; that is, crosses that possess androgenesis are processed via anther culture and the non-responsive ones are handled using conventional methods. Using this method, efforts are concentrated only on androgenetic material to increase greatly the efficiency. The present procedure consists of planting 15 F_1 plants 50 days prior to planting the F_1 crosses in the HBV evaluation nursery and anthers are plated only from R resistant plants from crosses possessing high levels of callus formation.

b. Development of Material for Cuba and the Southern Cone

Germplasm suitable for Cuba must be cold-tolerant, possess tolerance to Fe toxicity, resistance to blast and HBV. These characters exist in two distinct germplasms: cold tolerance from

the Chilean R_3 lines and the other characters from the lines listed in Table 10. Donors that possess the desired agronomic traits and also respond to anther culture will be crossed for processing via anther culture.

A similar procedure will be used for southern Brazil. Cold tolerance will be obtained from the Chilean material and Fe toxicity and blast tolerance from the upland dwarfs recently derived via anther culture. Again, all parents not only fulfill the agronomic requirement but also possess androgenetic properties. These crosses will be handled exclusively using anther culture.

The material presently being evaluated in Chile has the qualities to produce new commercial varieties for that country following local evaluation and seed multiplication. No new crosses are programmed until information is available concerning the performance of this material.

c. Irrigated Rice Program

The upland-dwarfs previously described are being incorporated into the irrigated crossing program to broaden the genetic base and to process selected crosses via anther culture.

d. Development of National Anther Culture Programs

Efforts have been made to strengthen facilities in Brazil. A small anther culture laboratory exists at the University of Pelotas, Rio Grande do Sul. Some of the crosses programmed for the

Southern Cone will be processed in this laboratory. However, infrastructure and personnel must be expanded if anther culture is to be used for breeding purposes.

Cuba also has expressed interest in establishing an anther culture facility. A Cuban scientist has received training in rice anther culture at CIAT.

ACKNOWLEDGEMENTS

Sincere appreciation is extended to Dr. Willy Roca, Head of Biotechnology Unit, as due to his efforts a rice culture program was established at CIAT. The assistance of Dr. John Miles and Dr. Roca in editing the manuscript is acknowledged. The valuable technical assistance provided by Mr. Victor Nuñez is appreciated. The author thanks Rosalba Reyes, Diego Macías, Nancy Coral, Gerardo Delgado, José F. Ortega and Omar Herrera for the long hours of work involved in this project. Special consideration is given to Dr. César Martínez, Dr. Surapong Sarkarung, and Mr. Julio Holguín for assisting in the planting and selection of the breeding material.