IAEA-TECDOC-859

# *Use of mutation techniques for improvement of cereals in Latin America*

*Final reports of a co-ordinated research programme*
*organized by the*
*Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture*

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Plant Breeding Section
International Atomic Energy Agency
Wagramerstrasse 5
P.O. Box 100
A-1400 Vienna, Austria

USE OF MUTATION TECHNIQUES
FOR IMPROVEMENT OF CEREALS IN LATIN AMERICA
IAEA, VIENNA, 1996
IAEA-TECDOC-859
ISSN 1011–4289



Printed by the IAEA in Austria
January 1996

# FOREWORD

This publication presents the scientific results obtained under the FAO/IAEA Co-ordinated Research Programme on Improvement of Cereals in Latin America through Mutation Breeding, in which breeders from Bolivia, Brazil, Chile, Colombia, Costa Rica, Cuba, Guatemala, Peru and Uruguay participated. This scientific network was logistically supported by the FAO/IAEA Regional Technical Co-operation Project (ARCAL VII) and many national FAO/IAEA Technical Co-operation Projects. In addition to the scientific results presented by the participants, this integrated approach of the IAEA produced significant plant breeding achievements. Several of them which are especially important for crop improvement in the region are described below:

- Bread wheat mutants with tolerance to aluminum toxicity, obtained after gamma irradiation (Brazil). This plant character is extremely important for increasing yields of wheat cultivated in large areas of Brazil as well as in other countries of the region, e.g. Colombia, Argentina and Peru.
- Mutants with increased tolerance to phosphorus deficiency in soils, selected and agronomically characterized in Chile.
- Early maturing mutants in local, well adapted varieties of rice. These mutations were obtained independently in Colombia, Costa Rica, Cuba and Guatemala and after seed irradiation with gamma rays. Rice earliness allows more productive utilization of farmers' fields in crop rotations in all these countries.
- Mutants with increased disease tolerance. Blast disease tolerance in rice was independently obtained following mutagenic treatments in Brazil, Costa Rica and Guatemala. Bread wheat mutants with rust tolerance were selected from irradiated plant materials in Brazil, Chile and Peru. In addition, resistance to *Septoria* was found in irradiated wheat in Brazil.
- Mutants with improved grain quality were selected from mutated progenies of barley and rice. Naked barley mutants, especially desired for human consumption in Peru, were frequently found in progenies of irradiated seeds of a local variety. Rice grain quality was improved in Brazil, Colombia and Cuba.
- Mutants with improved morphological characters such as leaf shape and especially semi-dwarfism, were selected in rice, barley and wheat in almost all countries participating in the project.

Some of the selected mutants were already tested in multilocation trials and had higher yields and/or other advantages, in comparison with the leading local varieties. On the basis of these results it is expected that a few new mutant varieties of rice (Brazil, Costa Rica, Cuba, Guatemala), wheat (Brazil, Chile) and barley (Peru) will be officially released within the next few years. Still more mutants, which are valuable for conventional breeding programmes as new sources of desired genes, were selected.

This TECDOC was jointly edited by A. Ashri, Israel, C. Bollich, USA, and the Scientific Secretary of the final research co-ordination meeting, M. Maluszynski.

## *EDITORIAL NOTE*

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## CONTENTS

# INTRODUCTION

Improvement of crop production by plant breeding is one of the activities that shows high returns and a very high cost/benefit ratio, in Latin America as well as in other parts of the world. From an agricultural point of view, this region is characterized by a wide diversity of crops, climatic conditions, soil types and specific production problems. Among the factors limiting crop production, the most important ones are salinity, drought, soil aluminum toxicity, deficiency of available phosphorous in the soil as well as susceptibility to diseases and pests. Food production in the region must be increased in order to meet the challenge of feeding its constantly growing population. Plant breeding provides the means to alleviate these problems and to achieve the desired goals by developing better adapted, more resistant and more productive varieties. All available modern tools must be used in order to implement successful breeding programmes and to increase the genetic variability necessary for the creation of new varieties. Conventional mutation techniques have been used often and in a wide array of crops to improve their adaptation, yield, quality, disease and pest resistance and to increase the attractiveness of flowers and ornamental plants. Mutation techniques have been used successfully in all parts of the world, resulting in the release of numerous (more than 1800) mutant varieties, directly or indirectly derived from the application of these techniques.

Current biotechnological approaches in combination with mutation techniques offer many possibilities to breed desired varieties in a relatively short time. Doubled haploids developed from anther, embryo or microspore culture are very suitable for radiation induced mutations and can speed up conventional breeding programmes in seed propagated crops. In order to establish facilities and to transfer to this region the doubled haploid and mutation techniques, the FAO/IAEA Regional Technical Co-operation Project (ARCAL VII) on Improvement of Rice and other Cereals through Mutation Breeding in Latin America was organized in 1985 and continued until 1993. On the basis of this activity the IAEA has subsequently established the Co-ordinated Research Programme (CRP) on Improvement of Cereals in Latin America through Mutation Breeding. This integrated approach of technical assistance and a research network proved successful and led to the transfer of these technologies and to the development of valuable breeding materials in important food crops such as rice, wheat and barley. The results of the research performed under this CRP and discussed at the final research co-ordination meeting are presented in this publication.

# BREEDING FOR GRAIN QUALITY AND EARLINESS IN RICE BY INDUCED MUTATIONS

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**Abstract**

The rice (*Oryza sativa* L.) breeding programme in the last 20 years has utilized natural variability derived mainly from hybridization. In 1989, a mutation breeding programme was initiated, in order to obtain new germplasm with improved characters such as milling quality, earliness, resistance to the "hoja blanca" virus disease and salt tolerance. Dry seeds of local variety Jucarito 104 (J-104) were treated with gamma rays (200 and 300 Gy) and neutrons (20 and 30 Gy). Finally 87 mutant lines were selected from the $M_5$ generation. They include 13 very early, 35 early and 39 medium maturing lines with slender, translucent grains. Some high yielding lines were also resistant to "hoja blanca" and had higher resistance to *Sogatodes* plant leafhoppers.

## 1. INTRODUCTION

Mutation breeding has become an established tool in plant breeding to supplement existing germplasm variability and to improve cultivars in certain specific traits [1]. In the last two decades great advances have been achieved in this field, and hundreds of mutants have been released as new cultivars. In rice, the results have been remarkable. Micke *et al.* [1] reported 251 released new rice varieties that were developed by the use of induced mutations. Of these, 167 were direct releases of mutants.

Rice is one of the most important crops in Cuba and is a staple food of the people. The rice breeding program in the last 20 years has utilized natural variability and artificial variability derived from hybridization. With the development of new, improved rice varieties, the breeding program has expanded to include mutation and tissue culture techniques. In 1989, a mutation breeding program was initiated, with the following objectives: (a) improving milling quality, (b) earliness, (c) resistance to the "hoja blanca" virus, and (d) salt tolerance. The present paper describes the results of research conducted in the period 1989-1991.

## 2. MATERIALS AND METHODS

An experiment was initiated in 1989 with the rice variety Jucarito 104 (J-104), the most popular variety in Cuba. It is semidwarf, and has a high yield potential, with excellent adaptability and stability and good resistance to the main pests and diseases. The principal disadvantages of J-104 are poor milling quality and a high number of chalky grains. The salient characteristics of this cultivar are given in Table I.

As a first step, the optimal radiation dose was determined for J-104. Two different sources of radiation were used: gamma rays from $^{60}Co$ and fast neutrons from a 14 MEV neutron generator. The radiosensitivity was determined by measuring the height of 21-day old seedlings derived from

irradiated seeds and those from non-treated seeds, and calculating the growth reduction rate. Radiation doses of 200 and 300 Gy for gamma rays and 20 and 30 Gy for fast neutrons with growth reduction of 10-20%, respectively, were selected for the breeding program. The outline of the breeding program is presented in Table II.

TABLE I. AGRONOMIC CHARACTERISTICS OF THE RICE VARIETY J-104 (CROSS IR480-5-93/IR930-1 6-1).

| Yield (t/ha) | | Maturity (days) | | Vg. | Lg. | Thr. | Disease/pest reaction | | | Grain quality | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Season | | Season | | | | | Hb | Bl | Sog | Head rice (%) | 1000 GW (g) | Clk |
| Dry | Wet | Dry | Wet | | | | | | | | | |
| 8.8 | 6.5 | 150 | 122 | 3 | R | MR | S | S | MR | 49 | 31.3 | 58 |

Vg - vigor; Lg - lodging; Thr - threshing; Hb - hoja blanca; Clk - translucence; R - resistant;
Bl - blast; Sog - *Sogatodes*; GW - 1000 grain weight; MR - moderately resistant; S -susceptible.

TABLE II. OUTLINE OF THE MUTATION BREEDING PROGRAMME WITH J-104.

| Season | Generation | No. of tested | | No. of selected | | Remarks |
|---|---|---|---|---|---|---|
| | | Lines | Plants | Lines | Plants | |
| 1989 D | $M_1$ | 0 | 9600 | 0 | 0 | Two panicles taken from each $M_1$ plant |
| 1989 W | $M_2$ | 9600 | 120 000 | 86 | 439 | Selection for earliness; slender and larger grains, and good plant type |
| 1990 D | $M_3$ | 439 | 0 | 215 | 1135 | Selection for earliness; slender and larger grains |
| 1990 W | $M_4$ | 215 | 1135 | 150 | 425 | Observational yield trials (pedigree rows); screening for Hb, Sog, grain quality |
| 1991 D | $M_5$ | 150 | 425 | 87 | 82 | Observational yield trials (pedigree rows) in three locations |
| 1991 W | $M_6$ | 121 | 48 | 0 | 0 | Multilocation trials |

D - dry season; W - wet season; Hb - hoja blanca; Sog - *Sogatodes*.

The irradiated ($M_1$) seeds were sown in a seed bed and transplanted to the field, with one plant per hill at a cross spacing of 15 x 15 cm. The first two panicles were harvested from each plant and the progeny from each $M_1$ panicle were raised as $M_2$ lines. The $M_2$ seeds were sown in a seed bed and the 21-day old seedlings were transplanted to a paddy field in progeny rows, with a single plant per hill within the rows. The hills were spaced 25 x 30 cm apart. One row of untreated seed was planted every 20 rows. A total of 30 000 plants per treatment were sown. At maturity, selections were made with the following objectives: long, slender grains; larger grains; earliness; and plant type similar to J-104 but slightly taller and compact.

One panicle of each $M_2$ plant was harvested for salt tolerance tests. The $M_3$ generation was directly seeded in the field in 5 m rows, with one row per selected $M_2$ plant. The rows were spaced 30 cm apart and the planting rate was 5 seeds per meter row. One row of untreated seed was planted every 20 rows.

The selection objectives were the same as with the $M_2$ generation. Five plants were harvested individually from each selected line, and the remaining plants in the uniform lines were harvested in bulk for observational trials. In segregating $M_3$ lines, individual plants were harvested and grown as pedigree rows in the following generation. All lines were screened for reaction to "hoja blanca" and the plant hopper *Sogatodes orizicola*, following the methodology of CIAT [2,3]. The following characters were evaluated according to IRRI standards: lodging, vigor, threshing, plant type and grain quality [4].

The $M_4$ and $M_5$ lines were sown in plots 8 rows x 5 m long, with 15 cm between rows. The seeding rate was 150 kg/ha. Two check plots were included every 10 plots, the original variety (J-104) and the early commercial variety Amistad 82. In the $M_5$ generation different trials for very early, early, and medium-late lines were conducted in three locations in the country. Recommended cultural practices were followed, according to the technical standards for rice in Cuba [5].

## 3. RESULTS

In the $M_1$ generation, germination and seedling survival were not affected by the radiation doses employed, and there was no detectable effect on the fertility of the $M_1$ plants.

Wide variation was observed in the $M_2$ generation, and plants were carefully screened for grain morphology and heading date. In this generation, a total of 439 plants with slender and translucent grains and or earliness were selected from 86 lines. Other types of mutations were observed, e.g., compact plants slightly taller than the control, bold grain types, late maturing types, and one line with semidwarf plants. From these, only ten plants from five strains of the compact plant type were selected. The number and types of plants selected are shown in Table III. The number of mutations from the gamma rays treatment was higher than from the neutron treatment, particularly mutations for grain shape. Early type mutations were identified in the neutron treated populations, some 20 to 25 days earlier than the parental variety. The occurrence of slender grain types was high, and 369 plants were selected. Of these, 16 possessed fine grains longer than the original parent. A large number of mutants combined fine grains with earliness.

In the $M_3$ generation, extensive variability was observed within some lines, primarily those from the gamma rays treatment. Also noted were reductions in heading date, in comparison with the $M_2$ generation. These findings may be due to a response of the mutants to stress caused by low temperatures that occurred during the season. In respect to grain shape, there was no difference between the generations and the lines showed good uniformity.

TABLE III. NUMBER AND TYPES OF MUTANTS SELECTED IN THE $M_2$ GENERATION IN THE WET SEASON.

| Treatment | No. of selected plants | No. of mutants with improved characters | | | | |
|---|---|---|---|---|---|---|
| | | Earliness (7-30 days*) | Grain shape | | | Compact plant type |
| | | | Slender | Slender longer | Larger | |
| Neutrons 20 Gy | 79 | 42 | 69 | 0 | 0 | 7 |
| Neutrons 30 Gy | 77 | 44 | 71 | 0 | 2 | 0 |
| Gamma rays 200 Gy | 133 | 24 | 109 | 0 | 4 | 2 |
| Gamma rays 300 Gy | 160 | 31 | 114 | 16 | 0 | 1 |
| Total | 449 | 141 | 363 | 16 | 6 | 10 |

*Days earlier than control

Table IV includes the numbers and types of mutant selections made in the $M_3$ generation. The observational yield trials were started with these selected lines. From the segregating lines, 291 plants were selected and further studies were done in pedigree rows.

TABLE IV. NUMBER AND TYPES OF MUTANT LINES SELECTED IN THE $M_3$ GENERATION IN THE DRY SEASON.

| Treatment | Mutant types and number | | | | | | | Total |
|---|---|---|---|---|---|---|---|---|
| | Days earlier than control | | | Grain shape | | | Compact plant type | |
| | 25-26 | 7-24 | 0 | Slender | Slender longer | Larger | | |
| Neutrons 20 Gy | 18 | 28 | 51 | 71 | 0 | 0 | 7 | 104 |
| Neutrons 30 Gy | 31 | 30 | 14 | 71 | 0 | 2 | 0 | 75 |
| Gamma 200 Gy | 0 | 6 | 1 | 16 | 0 | 2 | 2 | 16 |
| Gamma 300 Gy | 0 | 8 | 9 | 9 | 9 | 0 | 1 | 18 |
| | 49 | 72 | 84 | 167 | 9 | 4 | 10 | 213 |

Screening for "hoja blanca" and *Sogatodes orizicola* resistance was initiated in the $M_3$ generation. Based on two evaluations, one in the $M_3$ and one in the $M_4$ generation, 12 lines showed resistance to "hoja blanca" and 5 to *Sogatodes*, with 5 lines resistant to both. Another few lines gave a resistant reaction in only one test, and need to be tested further. The lines with resistance to both the virus and the insect are shown in Table V.

In the $M_4$ and $M_5$ observational trials, 87 lines were identified that combined the desirable characteristics of the parent variety like vigor, plant type, lodging resistance, and threshing resistance, with fine, translucent grains. Some were also very early, resistant to "hoja blanca", and had higher resistance to *Sogatodes*.

TABLE V. $M_4$ AND $M_5$ LINES RESISTANT TO HOJA BLANCA AND *Sogatodes orizicola.*

| Mutant line | Days to maturity | Reaction to | | | |
|---|---|---|---|---|---|
| | | Hoja blanca | | Sogatodes | |
| | | $M_4$ | $M_5$ | $M_4$ | $M_5$ |
| J104 N2-C26* | 120 | MR | R | MR | MR |
| J104 N2-C39 | 122 | MR | R | R | R |
| J104 N3-C126 | 122 | MR | MR | R | R |
| J104 N3-C78 | 126 | MR | MR | R | R |
| J104 G2-C267 | 138 | R | MR | MR | MR |
| J104 (Control) | 145 | S | S | MR | MR |

* N2 = Neutrons, 20 Gy; N3 = Neutrons, 30 Gy; G2 = Gamma rays, 200 Gy; R = Resistant; MR = Moderately resistant; S = Susceptible.

An additional 34 lines, from pedigree rows, have recently been added to the observational yield trials. Table VI includes the grain yield of the mutant lines in the observational trials. Overall, 5.7% of the lines yielded higher than the control, and 19.5% yielded 95 to 100% of the control yield. Many of the very early and early lines were inferior to the source variety in agronomic characteristics, but a few were superior. The lowest yields were observed in the very early or early mutants that were more than 20 days earlier than the control.

TABLE VI. RELATIVE GRAIN YIELD OF SELECTED MUTANT LINES FROM VARIETY J-104 IN $M_4$ AND $M_5$ GENERATION OBSERVATIONAL TRIALS.

| | Yield classes (% of control) | | | | | | | | | | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 50/65 | 66/70 | 71/75 | 76/80 | 81/85 | 86/90 | 91/95 | 96/100 | 101/105 | 106/110 | |
| No. of lines | 6 | 10 | 8 | 7 | 8 | 14 | 12 | 17 | 2 | 3 | 87 |

The performance of the best lines in the observational trials is presented in Table VII. All of the lines that yielded similar to or higher than the original parent were slightly earlier. As an exception, three very early mutants yielded similar to the control, J-104.

## 4. DISCUSSION

Early maturity and grain shape are two of the easiest mutations to detect by simple observations or measurements. Mutations for early maturity and grain shape also are characters for which mutations can be easily induced. Many major genes influence the expression of these characters, and most of the early maturing and fine grain mutants are recessive and show monogenic inheritance. In rice, several early maturing and fine grain mutants have been released directly as new cultivars. These findings were reported by different authors [1,6]. The results of our experiments

indicate that mutation breeding is an effective tool for improving heading date and grain morphology in rice, as has been reported by other authors [7,8,9].

TABLE VII. CHARACTERISTICS OF THE MOST PROMISING $M_5$ GENERATION MUTANT LINES IN OBSERVATIONAL TRIALS IN THE DRY SEASON.

| Mutant lines | Lodging | Cycle* (days) | Yield* (t/ha) | Reaction to Hb | Reaction to Sog | | 1000 grain wt. (g) | Chalk (%) |
|---|---|---|---|---|---|---|---|---|
| N2-C53-3 | R | -7 | +1.4 | MS | R | MR | 30.1 | 10.0 |
| N2-C59-2 | R | -5 | +1.2 | R | MR | R | 30.8 | 10.8 |
| N2-C59-5 | R | -7 | +1.0 | MR | MR | MR | 31.2 | 6.4 |
| N2-C72-2 | R | -9 | +0.3 | MR | MS | MR | 30.7 | 8.0 |
| N3-ClO7-2 | R | -5 | +0.3 | S | MR | MR | 29.9 | 11.2 |
| 63-C298-1 | R | -5 | 0 | S | MR | MR | 30.7 | 13.3 |
| N3-Cl 18-3 | R | -5 | 0 | S | MR | R | 31.2 | 10.6 |
| N2-C43-1 | R | -3 | 0 | S | MR | MR | 30.4 | 9.7 |
| 63-C291-5 | R | -7 | 0 | MR | MR | R | 29.8 | 9.8 |
| 62-C272-4 | R | -7 | 0 | S | R | MR | 30.1 | 9.2 |
| N2-C25-5 | R | -9 | 0 | MR | MR | MS | 29.3 | 11.6 |
| 63-C289-2 | R | -9 | 0 | R | MR | S | 29.7 | 10.7 |
| N2-259 | R | -7 | 0 | R | MR | MR | 29.4 | 9.3 |
| N2-C52-2 | R | -30 | - 0.3 | S | MR | MR | 32.2 | 9.7 |
| N3-s4-1 | R | -32 | - 0.5 | MR | MR | MS | 27.5 | 8.4 |
| 63-C289 | R | -28 | - 0.3 | R | MS | MS | 30.1 | 11.7 |
| J-104 (parent) | R | 0 | 0 | S S | MR | MS | 31.3 | 32.0 |

*Cycle and yield are expressed in differences from the original parent.
Hb = Hoja blanca; Sog = *Sogatodes orizicola.*

In our experiments, 87 mutant lines were selected for the last phase of the observational yield trials. They include 13 very early, 35 early, and 39 late maturing lines with slender, translucent grains. Some of them also combine resistance to the "hoja blanca" virus and higher resistance to *Sogatodes* than the parental variety.

It is important to note that the employment of gamma rays and neutrons with the same variety gave different mutations among the wide range of characters for which we were looking. The possibility of obtaining combinations of desirable characters is a very important reason for utilizing ionizing radiations of different LETs in a mutation breeding program.

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# INCREASING UPLAND RICE VARIABILITY THROUGH INDUCED MUTATIONS

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**Abstract**

Upland rice cultivars in Sao Paulo State, Brazil are generally tall (120-135 cm) even under low fertility soils ("cerrado"). In areas of continuous rice cultivation soil fertility is usually restored and the plants grow leafy, lodge, and seldom yield more than 2 t/ha. A mutation breeding programme involving Instituto Agronomico (IAC), International Atomic Energy Agency (IAEA), and Centro de Energia Nuclear na Agricultura (CENA) was started with the following objectives: (1) to induce a semidwarf mutant gene in the upland cultivar IAC 165; (2) once obtained, to incorporate the semidwarf gene in the standard hybridization program; and (3) to hybridize the new semidwarf source with the $sd_1$ gene in order to identify useful nonallelic semidwarf genes primarily for upland conditions. Fifty-five thousand seeds were treated with 40 and 45 krad (400 and 450 Gy). Conventional methods of handling and selecting mutant plants were carried out at the Campinas Experimental Center. Sixty-three mutant lines were selected and classified. In 1989 eight and in 1990 ten promising mutant lines were included in preliminary yield trials under upland conditions. In both years IAC 165 yielded more than the selected mutants. Under sprinkler irrigation several mutants yielded as high as IAC 165. Some mutants were later than IAC 165. Preliminary results of crosses involving the eight selected mutants with BR-IRGA 409 indicate that the mutant genes are not allelic to $sd_1$. Crosses of the mutants with IAC 165 indicate that the tall types are dominant.

## 1. INTRODUCTION

Rice is an important crop in Sao Paulo State and in all other States of Brazil. It is grown under at least four different systems. Of the total area of 5 278 000 hectares of rice grown in Brazil, 3 000 000 (58%) are in upland rice and 927 000 in paddy (irrigated) rice annually. Rice is a staple item in the diet of the Brazilian people and the per capita consumption is 73 kg/year. The country produces about 9 million tons annually, and the average yield is about 1500 kg/ha. In Sao Paulo State 70% of the rice area is in upland cultivars. About 10% of the area is in indigenous cultivars, mainly on small farms, and the remaining 90% is in released cultivars. Upland cultivars generally are tall (120-135 cm) and seldom yield more than 3000 kg/ha on low fertility soils (cerrado). Usually, after growing rice continuously the soil fertility level improves and the cultivars tend to grow leafy and tall.

Over the last decade mutation techniques have become one of the most important tools available to progressive plant breeding programmes [1]. In California induced and spontaneous rice mutants have not only been released directly as improved cultivars but, more importantly, they have also been used as donor parents in standard cross breeding programmes [2,3].

No upland semidwarf rice cultivars are grown in Brazil. Only paddy cultivars include semidwarf types. The upland rice breeding programme has used some semidwarf donors of the tropical indica type, mainly from the International Rice Research Institute (IRRI) and Centro Internacional de Agricultura Tropical (CIAT), in crosses with local upland cultivars. The local cultivars do not have acceptable plant types and considerable backcrossing was required to obtain satisfactory adaptation in the segregating populations. Because of these problems a new approach

involving IAC, CENA, and the IAEA was initiated in 1986-87 to obtain a semidwarf mutant from the principal upland cultivar, IAC 165. The objectives were: a) to induce a semidwarf mutant for release as a new cultivar; b) to hybridize any newly-induced semidwarf with the $sd_1$ gene to identify useful nonallelic semidwarf mutant genes; c) to hybridize any semidwarf sources obtained through mutation techniques with the indigenous and other local upland varieties in the cross breeding programme.

## 2. MATERIALS AND METHODS

Seeds of the cultivar IAC 165, which originated from the cross IAC 1246 X Dourado Precoce, were equilibrated to a moisture level of approximately 12% and treated with $^{60}Co$ at the Centro de Energia Nuclear na Agricultura, Piracicaba. After a preliminary test to establish the dosages of 40 and 45 krad, approximately 55 000 seeds from each treatment were planted in rows spaced 0.60 m apart. Every 20th row was a check row. In the same year, 2436 panicle rows of the selected $M_1$ panicles for both the 40 and 45 krad (D1 and D2) populations were grown. Each $M_2$ progeny was manually seeded in the field at the Experimental Station in Campinas in a row 5 m long. Each row contained about 30 plants. Selection was based on plant height as compared with the check. Each $M_2$ plant that contained a desired mutant was continued as an $M_3$ line. When mutants were harvested in a segregating line, normal plants were also harvested in order to conduct a progeny test of heterozygous sister plants in the $M_3$.

Sixty-three short stature mutants were grown in 1989 to obtain data on plant height and days to flowering. In the previous generation the tendency of the mutants was to flower later than IAC 165. Preliminary yield trials of eight promising mutant lines and the control were conducted at Campinas in order to check the yield potential of the mutants in a randomized complete block design with four replications. At the same time, tolerance of the mutants to aluminum toxicity was evaluated in a nutrient solution.

In 1989 the mutants "M 1" to "M 9" were crossed with the parent cultivar IAC 165 and with the semidwarf cultivar BR IRGA 409, which carries the $sd_1$ gene. In 1990 the $F_1$ plants of all of the crosses and the parents were grown in the experimental field at Campinas at a spacing of 15 X 30 cm. The $F_1$ plants were grown adjacent to their respective parents. Plant height and panicle length of 10 plants per line were measured at maturity.

## 3. RESULTS AND DISCUSSION

*Determination of gamma rays sensitivity*

Table I presents seedling height for irradiated IAC 165 at 15 days after emergence in relation to the control, which is considered to be 100%. As expected, seedling height decreased as dosage increased. IAC 165 exhibited appreciable sensitivity at 30 krad. Doses of 40 and 45 krad were selected for the project. In the $M_1$ generation an interaction was observed between irradiation effects and environmental conditions.

*Obtaining the $M_2$ generation*

In the 2436 and 2649 $M_1$ panicle rows at the lower and higher dose rates, respectively, 167 lines (6%) segregated for albinism at the 45 krad dose (801 albinos in a total of 4611 plants). At the 40 krad dose, only 16 lines (1%) segregated for albinism (106 albinos in a total of 525 plants). At maturity, 17 lines at the lower dose and 46 lines at the higher dose segregated for plant height.

TABLE I. PRELIMINARY RADIATION DOSE TEST FOR IAC 165 RICE VARIETY, BASED ON PLANT HEIGHT.

| Radiation dose (krad) | Plant height in percent of control* |
|---|---|
| 10 | 100.0 |
| 20 | 99.3 |
| 30 | 89.1 |
| 40 | 77.5 |
| 50 | 53.6 |

*Fifteen days after emergence.

*Selection in $M_2$ to $M_5$ generation*

After selection for semidwarfism on an individual basis, the semidwarf mutants were classified for other phenotypic characters (Table II). The 45 krad dose induced a greater number of short stature mutants than the 40 krad dose. Eighteen mutants were shorter than 80 cm, 8 were taller than 100 cm, which was almost the same height as that of IAC 165 (109 cm), and the remainder were between 80 and 100 cm, the desirable height range.

A great majority of the mutants (51) flowered later than the parent variety. Those that flowered as early as IAC 165 were derived from the 45 krad dose.

In respect to phenotypic characteristics, the selected mutants showed the same behavior as the parent variety. Thirty-three had a phenotypic appearance classified as fair; 35 were medium tillering; 57 had an intermediate panicle type; 42 had intermediate type leaves; 49 exhibited good panicle exsertion; 56 had a medium grain shape; and 48 had the same disease reaction as IAC 165. Seven mutants manifested good disease reaction or field tolerance to neck blast (*Pyricularia grisea*). One mutant line had a slender grain shape. It should be noted that a majority of the mutants were derived from the 45 krad dose population. Table III summarizes the data for plant height, days to flowering, and yield of 18 mutants at the Experimental Station in Campinas in 1989. In both years IAC 165 produced higher yields than the selected mutants. In the 1989 yield trial, which included 8 mutants and the IAC 165 check, only one mutant showed a fair production. It had semidwarf plant height and was as early maturing as IAC 165. The remaining 7 mutants showed unsatisfactory field performance.

The 1990 yield trial included 10 mutants and IAC 165. The 10 mutant lines were selected from the 63 previously selected mutants. The yield trial was sown at a 40 cm row spacing and received supplementary irrigation. Two mutants yielded as well as IAC 165 but neither possessed semidwarf height and one was 6 days later than IAC 165. The later mutant was discarded because the later maturity is unacceptable for upland conditions in Sao Paulo State. Only one semidwarf mutant, "M 14", showed a good yield response associated with earliness (Table IV).

The preliminary results of the crosses involving the 8 selected mutants with BR IRGA 409 (Fig. 1) indicates that the genes are nonallelic to the $sd_1$ gene and they may constitute a new genetic source for semidwarfism. Figure 2 presents the plant height data for the parents and $F_1$ generation of the crosses of the 8 mutants and BR-IRGA 409. The dominance of the tall type is evident.

TABLE II. PHENOTYPIC CHARACTERISTICS OF SELECTED RICE MUTANTS.

| Character | Mutant evaluation | Control evaluation | Number of mutants | | |
|---|---|---|---|---|---|
| | | | 40 krad | 45 krad | Total |
| Plant height | < 80 cm | | - | 12 | 18 |
| | 81-90 cm | | - | 15 | 16 |
| | 91-100 cm | | - | 12 | 21 |
| | > 100 | IAC 165 | - | 7 | 8 |
| Days to flower | < 80 days | IAC 165 | - | 12 | 12 |
| | 80-90 days | | - | 23 | 39 |
| | > 90 days | | 1 | 11 | 12 |
| Phenotypic appearance | Poor | | 3 | 19 | 22 |
| | Fair | IAC 165 | 13 | 20 | 33 |
| | Good | | 1 | 7 | 8 |
| Tillering | Poor | | 3 | 5 | 8 |
| | Medium | IAC 165 | 13 | 22 | 35 |
| | Good | | 1 | 19 | 20 |
| Panicle type | Compact | | 3 | 2 | 5 |
| | Intermediate | IAC 165 | 14 | 43 | 57 |
| | Open | | - | 1 | 1 |
| Spikelet sterility | Fertile | IAC 165 | 9 | 36 | 45 |
| | Sterile | | 8 | 10 | 18 |
| Panicle exsertion | Poor | | 2 | 5 | 7 |
| | Fair | | 3 | 4 | 7 |
| | Good | IAC 165 | 12 | 37 | 49 |
| Grain shape | Bold | | 3 | 3 | 6 |
| | Medium | IAC 165 | 14 | 42 | 56 |
| | Slender | | - | 1 | 1 |
| Leaf width | Narrow | | - | 5 | 5 |
| | Intermediate | IAC 165 | 13 | 39 | 52 |
| | Wide | | 4 | 2 | 6 |
| Disease reaction | Poor | | 1 | 7 | 8 |
| | Fair | IAC 165 | 15 | 33 | 48 |
| | Good | | 1 | 6 | 7 |

## 4. CONCLUSIONS

The 45 krad irradiation dose caused a greater frequency of short statured mutants compared with 40 krad and increased the frequency of undesirable types. It is expected that more promising material will be obtained from segregating populations of mutant crosses with the parent varieties. Additionally, it should be considered that in evaluating the performance of semidwarf mutants, yield trials must be adapted to the architecture of semidwarfs and full consideration given to row spacing, soil fertility, and sprinkler irrigation when feasible. The great majority of mutants flowered later than IAC 165.

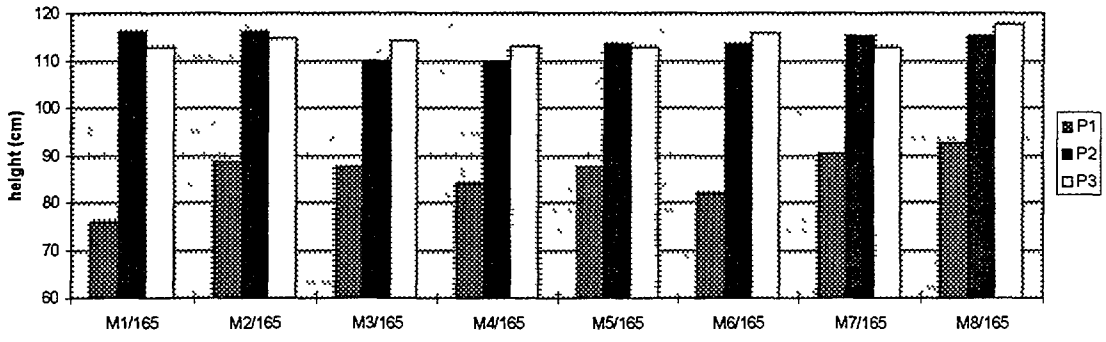


*FIG.1. Plant height of parents and F1 crosses involving 8 mutants and the parent variety cultivar IAC 165.*

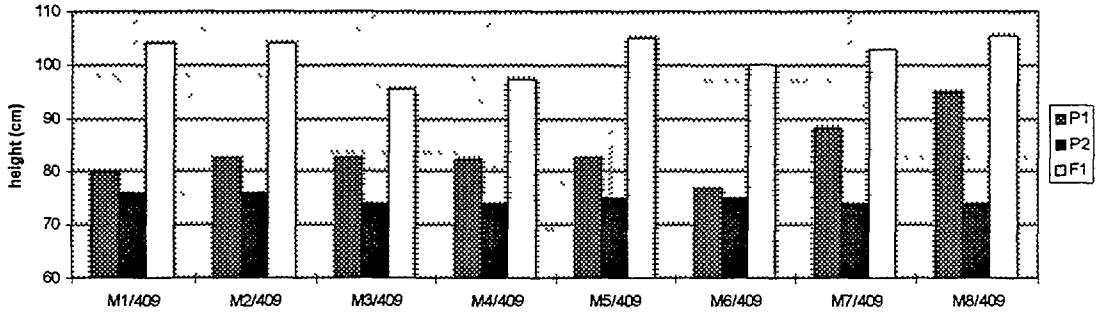


*FIG 2. Plant height of parents and F1 crosses involving 8 mutants and Br-IRGA 409.*

TABLE III. DEVELOPMENTAL TRAITS AND GRAIN YIELD OF EIGHT SELECTED RICE MUTANTS AND IAC 165, CAMPINAS, 1989.*

| Cultivar and mutants (cm) | Plant height (cm) | Panicle length (days) | Days to flower (No.) | Grain yield (kg/ha) |
|---|---|---|---|---|
| IAC 165 | 110 a | 21.2 | 76 | 3969 a |
| M 1 | 75 c | 21.9 | 80 | 1586 c |
| M 2 | 90 b | 17.3 | 83 | 1672 c |
| M 3 | 85 b | 15.4 | 76 | 2400 b |
| M 4 | 89 b | 18.1 | 94 | 1350 c |
| M 5 | 90 b | 19.6 | 78 | 1628 c |
| M 6 | 86 b | 16.9 | 82 | 1422 c |
| M 7 | 92 b | 18.2 | 86 | 1778 c |
| M 8 | 86 b | 18.2 | 86 | 1583 c |

*Within columns, values followed by different letters are significantly different at the 5% level.

$F_1$ data from hybrids of crosses of 8 short-statured mutants with BR-IRGA 409, which carries the $sd_1$ gene, indicate that the mutant genes are nonallelic to $sd_1$.

Induced mutations can be an effective breeding tool in achieving specific objectives that involve readily identifiable qualitative recessive traits.

TABLE IV. DEVELOPMENTAL TRAITS AND GRAIN YIELD OF 10 SELECTED RICE MUTANTS AND IAC 165, CAMPINAS, 1990.

| Cultivar and mutants (cm) | Plant height (cm) | | Days to flower (No.) | Grain yield (kg/ha) | |
|---|---|---|---|---|---|
| IAC 165 | 103 | a | 80 | 3067 | a |
| M 9 | 92 | cd | 86 | 1111 | bc |
| M 10 | 78 | ef | 82 | 1956 | abc |
| M 11 | 100 | bc | 86 | 3102 | a |
| M 12 | 75 | f | 76 | 2280 | abc |
| M 13 | 113 | a | 81 | 3000 | a |
| M 14 | 93 | bcd | 80 | 2951 | a |
| M 15 | 87 | de | 83 | 2378 | abc |
| M 16 | 78 | ef | 83 | 1578 | bc |
| M 17 | 95 | bcd | 80 | 2622 | ab |
| M 18 | 95 | bcd | 83 | 2733 | ab |

*Within columns, values followed by different letters are significantly different at the 5% level.

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# MUTATION BREEDING FOR IRRIGATED RICE AT EMPASC, SANTA CATARINA, BRAZIL

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**Abstract**

Mutation breeding for irrigated rice culture was initiated in 1985 at Estacao Experimental de Itajai (EMPASC), Santa Catarina in cooperation with Centro de Energia Nuclear na Agricultura (CENA), Piracicaba, Sao Paulo. The traditional breeding programme conducted by EMPASC at Itajai is primarily based on the introduction of cultivars, pure lines, and hybridization. The new introductions have not been as good as the cultivars now grown by rice farmers.

Rice breeders are constantly attempting to develop new cultivars for irrigated rice culture and seeking new methods to supplement the conventional breeding methods. The introduction of mutation techniques to the breeding programme at Itajai began with the irradiation of seeds of 4 rice cultivars with 12 and 24 kr (120 & 240 Gy) of gamma rays at CENA in 1985. The irradiated seeds were sown at Itajai in the same year. Seeds were harvested from 3 panicles of each plant. The mutant plants were then cultivated to develop select progenies. Mutants were visible only in the cultivars IRGA 408, EMPASC 105, and Pratao Precoce. Fifty-seven progeny were selected primarily from IRGA 408 and EMPASC 101 for the $M_4$ generation. Progeny from Pratao Precoce were eliminated because they were not stable. Mutant progeny from EMPASC 101 were eliminated in the $M_5$ generation because of a high incidence of chalk in the rice grains. The agronomic performance of IRGA 408 progeny was good and some mutant lines were further evaluated as follows: one in regional trials, 5 in advanced trials, and 5 in preliminary trials. Mutant line SCM-3-1-2 was evaluated in a regional trial and showed strong promise of becoming a new cultivar.

## 1. INTRODUCTION

Research on irrigated rice first started in Santa Catarina state in Brazil. In the beginning, traditional cultivars and lines were introduced and their agronomic performance evaluated. Several cultivars were recommended for commercial production under irrigation. After 1969, new lines with modern plant types began to be tested. The main characteristics of the new genotypes were reduced height, high tillering, and high yield potential. In 1976/77 some of the modern types of cultivars were recommended, including EMPASC 101, EMPASC 102, IR841, CICA 4 and IR665.

Following the recommendation and production of the new varieties, problems were experienced in harvesting and commercialization mainly because of the plant type and grain size. Hand harvesting was difficult because of the short height of the plants. Processing (milling) was adversely affected by the difference in the grain size and shape, long and slender for the modern cultivars and long and thick for the traditional cultivars. Production of the modern cultivars was insufficient to justify separate processing and there was no interest in searching for new markets. However, these difficulties were not enough to discourage the production of the new cultivars, which were more productive than the traditional cultivars [1]. Because of the increasing numbers of producers growing the modern cultivars, mechanical harvesting was adopted and processing methods and industrial income were changed [2].

Two American cultivars, Dawn and Labelle, were recommended in 1978 because of their excellent grain quality and, in the case of Labelle, short growing cycle. Labelle was recommended for those growers that grew two crops on the same area in the same season [3]. In 1981, the line P-791-B4-14 was recommended under the name EMPASC-103.

At present in Santa Catarina state, all irrigated rice cultivars are the modern type and the prevailing cultivars vary with the production area. With the adoption of modern cultivars and the simultaneous production of high quality pure seed, the rice industry in Santa Catarina state entered a new phase after 1976/77.

The rice breeding program of EMPASC is currently based on introduction of genotypes, hybridization and mutations. Mutation techniques have been added to the program to broaden the variability of the rice germplasm, particularly for grain quality, earliness, and disease resistance.

## 2. MATERIALS AND METHODS

In 1985, seeds of the rice cultivars EMPASC 101, IRGA 408, Pratao Precoce, and CICA 8 were irradiated with 12 and 24 kr of gamma rays at CENA and the $M_1$ generation was sown at the Itajai Experimental Station. The seeds were sown in boxes and when the seedlings were two weeks old they were transplanted in the field by hand. Plants were spaced 20 cm x 30 cm.

$M_1$ - Three seeds were harvested from 3 panicles of each plant.

$M_2$ - In the 1986/87 cycle, the harvested seeds were sown to produce the population from which to select mutants.

$M_3$ - In the 1987/88 cycle, seeds from the mutant plants identified in the $M_2$ generation were sown and new plants were selected from segregating lines.

$M_4$ - In the 1988/89 cycle, 57 mutant lines were evaluated in a replicated test to compare their performance with that of the parent cultivar. In yield trials, the plots were broadcast-seeded with pre-germinated seed.

$M_5$ - In the 1989/90 cycle, selected lines from the $M_4$ generation were evaluated in a preliminary trial and the best lines were also evaluated in an advanced trial at Itajai.

$M_6$ - The best lines from the preliminary trial were evaluated in an advanced trial and those that had been evaluated in the advanced trial the previous year were evaluated in regional trials. The advanced trial was conducted in 5 ecological regions, namely, Pouso Redondo, Massaranduba, Itajai, Tubarao, and Turvo.

## 3. RESULTS AND DISCUSSION

*$M_2$ generation*

The $M_1$ seeds were bulk harvested and from the $M_2$ generation 20 plants were identified as possible mutants. Selection was based on visual observations relative to the parent cultivar. No mutants were identified in CICA 8.

*Preliminary evaluation of genotypes in the $M_3$ generation*

As described above, 20 mutants were selected for the $M_3$ generation. Three of the mutants were from EMPASC 101, 2 from Pratao Precoce, and 17 from IRGA 408. The irradiation dose of 12 kr was more effective than 24 kr in producing mutants.

Table I presents data from the $M_3$ generation and illustrates how subprogenies were derived from progenies. Line SCM-1-2 was the earliest and the shortest plant selected from EMPASC 101. Mutant genotypes from Pratao Precoce were not segregating, therefore 13 plants were selected to form subprogenies. The life cycle of the mutant genotypes from IRGA 409 varied between 112 and 140 days and the height from 93 to 118 cm. To increase the number of genotypes, 52 plants were selected to form new progeny.

TABLE I. AGRONOMIC CHARACTERISTICS OF INDUCED $M_3$ MUTANT GENOTYPES OF IRRIGATED RICE AT ITAJAI, SANTA CATARINA STATE, 1987/88.

| Genotype | Life* cycle (days) | Plant height (cm) | Milling yield (%) | Selected sub-progenies (number) |
|---|---|---|---|---|
| SCM-1-1** | 125 | 117 | 67.0 | 10 |
| SCM-1-2 | 109 | 82 | 67.0 | 1 |
| SCM-2-1 | 135 | 104 | 69.1 | 1 |
| EMPASC-101 (check) | 130 | 114 | 68.1 | - |
| SCM-5-1 | 109 | - | - | 7 |
| SCM-5-2 | 109 | - | - | 5 |
| SCM-6-1 | 109 | - | - | 1 |
| PRATÃO PRECOCE (check) | 109 | - | - | - |
| SCM-3-1 | 140 | 113 | 71.3 | 2 |
| SCM-3-2 | 130 | 104 | 70.0 | 1 |
| SCH-3-3 | 140 | 106 | 72.3 | 1 |
| SCM-3-4 | 140 | 97 | 72.3 | - |
| SCM-3-5 | 130 | 100 | 71.3 | 1 |
| SCM-3-6 | 140 | 102 | 70.9 | - |
| SCM-3-7 | 140 | 100 | 72.8 | - |
| SCM-3-8 | 114 | 95 | 63.1 | 1 |
| SCM-3-9 | 135 | 98 | 63.2 | 3 |
| SCM-3-10 | 130 | 118 | 70.8 | 10 |
| SCM-3-11 | 135 | 96 | 71.6 | - |
| SCM-3-12 | 140 | 97 | 71.4 | - |
| IRGA-408 (check) | 112 | 90 | 70.0 | - |
| SCM-4-1 | 135 | 96 | 69.3 | 1 |
| SCM-4-2 | 112 | 108 | 72.2 | 6 |
| SCM-4-3 | 140 | 103 | 68.7 | - |
| SCM-4-4 | 112 | 93 | 70.6 | 1 |
| SCM-4-5 | 130 | 103 | 70.4 | - |

* Number of days from germination till harvest.

** SCM-1-n was derived from EMPASC 101 irradiated with 12 krad.
SCM-2-n was derived from EMPASC 101 irradiated with 24 krad.
SCM-3-n was derived from IRGA 408 irradiated with 12 krad.
SCM-4-n was derived from IRGA 408 irradiated with 24 krad.
SCM-5-n was derived from Pratão Precoce irradiated with 12 krad.
SCM-6-n was derived from Pratão Precoce irradiated with 24 krad.

*Evaluation of mutant lines in the $M_4$ generation*

Fifty-seven mutant progenies were evaluated in the $M_4$ generation, primarily from IRGA 408 and EMPASC 101. The mutant lines from Pratao Precoce were eliminated because they were segregating for plant height and internode elongation and most plants were susceptible to lodging. In

the $M_4$ generation, 42 and 15 progeny, respectively, from IRGA 408 and EMPASC 101 were maintained for further screening.

Agronomic and grain quality characteristics of the mutant lines from IRGA 408 and EMPASC 101 are presented in Table II. The life cycle of the mutant lines derived from IRGA 408 were shorter than those derived from EMPASC 101 but longer than that of the parent cultivar IRGA 408. However, one mutant, SCM-4-2-3, from IRGA 408 grew faster than IRGA, maturing in 106 days. Six mutant lines had the same cycle. The cycle of the mutants obtained from EMPASC 101 was close to that of EMPASC 101. The best mutant, SCM 3-10-1, yielded 9.86 t/ha, which was 62% higher than that of the parent cultivar, IRGA 408. The lowest yielding mutant from IRGA 408 was SCM 3-8-1, which produced 5.34 t/ha, 13% lower than that of the parent cultivar. The mutant lines from EMPASC 101 did not yield as well as those from IRGA 408. The best yield, 8.58 t/ha, was produced by SCM 1-1-5 and was 17.1% higher than that of EMPASC 101. The poorest yield, 5.66 t/ha, was that of SCM 1-2-2.

TABLE II. GRAIN YIELD AND AGRONOMIC CHARACTERISTICS OF $M_6$ GENOTYPES EVALUATED IN ADVANCED TRIALS, EMPASC/EEI, 1990/91.

| Line or cultivar | Yield (t/ha) | Life cycle (days) | Plant height (cm) | 1000 grain weight (g) | Sterility (%) | Grain length (mm) | Grain width (mm) | Chalkiness (0-5) |
|---|---|---|---|---|---|---|---|---|
| P4473-F3-2-4-2-M-M | 7.93 | 137 | 89 | 30 | 32 | 6.5 | 2.3 | 3 |
| SCM 4-3-1 | 7.47 | 128 | 88 | 23 | 17 | 6.5 | 2.2 | 2 |
| SCM 3-1-2 | 8.52 | 119 | 88 | 31 | 15 | 7.0 | 2.3 | 2 |
| SCM 3-2-2 | 7.97 | 134 | 91 | 25 | 29 | 6.6 | 2.1 | 1 |
| SCM 3-9-2 | 7.66 | 127 | 85 | 31 | 23 | 7.0 | 2.3 | 2 |
| SCM 3-1-1 | 8.51 | 119 | 88 | 30 | 11 | 6.9 | 2.3 | 2 |
| CT 6919-3-5-2-2-M | 9.32 | 118 | 98 | 26 | 12 | 6.5 | 2.1 | 2 |
| EMPASC-105 | 9.38 | 121 | 88 | 32 | 11 | 6.8 | 2.3 | 1 |
| CICA-8 | 9.37 | 137 | 90 | 27 | 15 | 6.7 | 2.2 | 2 |
| BR-IRGA-409 | 7.31 | 106 | 105 | 30 | 14 | 6.6 | 2.1 | 2 |
| CNA 1214-BM-B-14-A | 8.80 | 129 | 99 | 30 | 29 | 7.7 | 2.2 | 4 |
| EMPASC-101 VELHO TURVO | 7.44 | 123 | 89 | 30 | 15 | 7.0 | 2.3 | 3 |
| EMPASC-101 NOVO TURVO | 8.28 | 122 | 99 | 32 | 17 | 7.1 | 2.2 | 4 |
| IAC 4440 A1 | 8.00 | 132 | 89 | 23 | 13 | 6.6 | 2.2 | 2 |
| CNA 5188-1 | 8.41 | 128 | 88 | 24 | 13 | 6.7 | 2.2 | 3 |
| EMPASC-101 VELHO COMBORIU | 8.47 | 125 | 91 | 28 | 21 | 6.8 | 2.3 | 3 |
| EMPASC-101 | 7.87 | 122 | 96 | 32 | 13 | 7.1 | 2.3 | 4 |
| CNA 1214-BM-B-14-B | 7.13 | 118 | 101 | 29 | 19 | 7.5 | 2.1 | 1 |

Grains from the mutants from EMPASC 101 usually exhibited "white belly" chalk. The best mutants that were free of chalk were derived from IRGA 408. Milling yields were similar among all the mutants and the parent cultivars, EMPASC 101 and IRGA 408. The exceptions were SCM 1-1-4, with only 61.19% total milled rice, and SCM 2-1-1 with 72.42% total milled rice.

The mutants SCM-3-11-1 and SCM-3-4-1 from IRGA 408 were selected for evaluation in the advanced trial. Selected for the preliminary trial were SCM-3-3-1, SCM-3-6-1, SCM-3-2-1, SCM-3-10-1, SCM-3-1-2, SCM-3-2-2, SCM-3-5-2, SCM-3-9-3, SCM-3-9-2, SCM-3-1-1, SCM-3-9-1, SCM-3-12-1, SCM-3-7-1, SCM-4-3-1, and SCM-4-1-1.

*Evaluation of mutant lines in the $M_5$ generation*

On the basis of agronomic data from the $M_5$ preliminary yield trial that included 15 genotypes derived from the mutation breeding programme and 23 genotypes that were developed by traditional breeding methods at Itajai, and additional data, the following genotypes were selected for the advanced trial: P4473-F3-2-4-2-M-M, SCM-4-3-1, SCM-3-1-2, SCM-3-2-2, SCM-3-9-2, SCM-3-1-1 and CT-6919-3-5-2-2-M. Grain yield, life cycle, plant height, and grain chalkiness were the principal characteristics emphasized in selecting lines for further testing but resistance to diseases and iron toxicity were also important. Some genotypes that produced acceptable yields were not selected because of weaknesses in other characteristics.

*Evaluation of mutant lines in the $M_6$ generation*

Five induced-mutant lines promoted from the 1989/90 preliminary trial were evaluated in the advanced trial. As shown in Table II, the grain production of CT 6919-3-5-2-2-M was similar to the CICA 8 and EMPASC 105 checks. All of the other lines had lower yields than both check cultivars but higher than the original IRGA 408 cultivar. All lines matured later than BR-IRGA 409. Two of them were similar to CICA 8 in maturity and 5 were similar to EMPASC 105. Three genotypes averaged more than 20% sterility but they yielded well, nonetheless. Overall, the best genotypes in the advanced yield trial were P4473-F3-2-4-2-M-M, SCM-3-1-2, SCM-3-2-2, SCM-3-1-1 and CT 6919-3-5-2-2-M. They were to be evaluated in the regional trials in the following year.

Table III presents the grain yields in the regional trials conducted in 1989/90 and 1990/91 at 5 locations in Santa Catarina State. The relatively low yields at Tubarao in 1990/91 resulted from late sowing, December 21. A water shortage prevented flooding the soil. The late sowing reduced yields more for the later maturing than for the early maturing cultivars.

The mutant line SCM 3-1-2 (code SC-117) produced very good yields in Itajai, Massaranduba, and Turvo. As shown in Table IV, which includes grain quality and agronomic data, SC-117 (SCM 3-1-2) generally was better than the check cultivars in respect to chalkiness, leaf blight, panicle neck blight, and lodging. It was more susceptible to iron toxicity than most of the standard cultivars, but was similar in reaction as BR-IRGA 409 and EMPASC 105.

Several early maturing lines, namely, SC 110, SC 100, BR-IRGA 414, and SC 111, produced good yields. At sites with a high iron concentration, SC 110 and SC 111 showed iron toxicity symptoms. The average yield of SC 100 (CT 7363-13-5-7-M), 6.5 t/ha, was similar to that of the standard cultivars and it is more resistant to iron toxicity, lodging, leaf blight, and panicle neck blight.

SC 117, which is intermediate in maturity (120 days), had an average yield of 7 t/ha. The late maturing line SC 2 had a higher average yield than CICA 8 and, in spite of phenotypic similarities, showed more lodging resistance.

## 4. CONCLUSIONS

Irradiation of seeds of IRGA 408 and IMPASC 101 increased genetic variability and produced a number of mutants in respect to the growth cycle, plant height, lodging resistance, reaction to iron toxicity, grain quality factors, plant architecture, disease reaction, and grain yielding ability. Several of the mutant lines derived from IRGA 408 performed very well relative to the parent variety for one or more characters and offer the potential for release as new cultivars or for use as parents in the

traditional breeding program. All of the mutant lines derived from EMPASC 101 were eliminated because of excessive grain chalkiness.

TABLE III. GRAIN YIELD (t/ha) OF IRRIGATED RICE CULTIVARS AND ADVANCED MUTANT LINES AT FIVE LOCATIONS IN SANTA CATARINA STATE, EMPASC/EEI, 1989 AND 1990.

| Line or cultivar | Code | Itajai | | Massaranduba | | Pouso Redondo | | Tubarao | | Turvo | | Mean |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1989 | 1990 | 1989 | 1990 | 1989 | 1990 | 1989 | 1990 | 1989 | 1990 | |
| CT-7363-13-5-1-M | SC-97 | - | 7.27 | - | 5.85 | - | 7.50 | - | 5.31 | - | 8.38 | 6.86 |
| 1RGA 177-F4-SS-11 | SC-110 | 6.34 | 7.54 | 6.68 | 5.63 | 7.36 | 6.85 | 5.51 | 5.13 | 4.89 | 7.05 | 6.30 |
| CT 7363-13-5-7-M | SC-100 | 7.38 | 8.05 | 6.05 | 5.86 | 6.90 | 7.47 | 3.95 | 5.46 | 5.44 | 8.38 | 6.50 |
| BR-1RGA-414 | - | 5.62 | 8.15 | 5.00 | 6.07 | 6.55 | 6.41 | 6.75 | 4.44 | 4.82 | 6.18 | 6.18 |
| CT 7363-13-5-6-M | SC-99 | - | 8.18 | - | 5.78 | - | 5.57 | - | 5.30 | - | 7.93 | 6.55 |
| P798 L 386-5-17 | SC-111 | 7.30 | 7.99 | 5.67 | 6.29 | 5.47 | 5.73 | 6.49 | 5.01 | 5.94 | 8.55 | 6.44 |
| P798 L 386-5-1 | SC-71 | 5.72 | 7.64 | 6.20 | 6.00 | 6.71 | - | 7.23 | 4.57 | 5.33 | 4.58 | 6.00 |
| CT 7363-13-4-5-M | SC-96 | 6.46 | 7.11 | 6.18 | 6.56 | 6.12 | 7.15 | 6.53 | 5.04 | 4.96 | 7.54 | 6.37 |
| CT 7363-17-1-2-M | SC-104 | - | 7.30 | - | 6.37 | - | 5.89 | - | 4.59 | - | 7.88 | 6.41 |
| CT 7363-17-2-1-M | SC-105 | - | 7.06 | - | 5.78 | - | 6.16 | - | 4.80 | - | 8.86 | 6.59 |
| CT 7363-13-6-2-M | SC-101 | - | 6.71 | - | 6.54 | - | 6.23 | - | 5.13 | - | 7.88 | 6.58 |
| SCM 3-1-2 | SC-117 | - | 8.20 | - | 8.53 | - | 6.73 | - | 4.51 | - | 8.95 | 7.38 |
| EMPASC-105 | - | 9.26 | 9.28 | 8.05 | 7.90 | 9.01 | 7.52 | 8.27 | 5.12 | 7.36 | 8.12 | 8.09 |
| CNA 5259 | SC-24 | 6.86 | 7.35 | 6.26 | 6.73 | 7.21 | 7.72 | 6.54 | 4.80 | 6.28 | 6.85 | 6.66 |
| P 2015-F4-54-1B-1B | SC-2 | - | 8.94 | - | 8.36 | - | 7.96 | - | 4.50 | - | 9.22 | 7.80 |
| CICA-8 | - | 9.07 | 8.53 | 7.04 | 7.80 | 8.83 | 7.58 | 5.56 | 4.58 | 8.23 | 9.17 | 7.64 |
| EMPASC-104 | - | 8.20 | 9.83 | 7.87 | 6.37 | 7.08 | 7.33 | 7.76 | 4.94 | 7.37 | 8.99 | 7.57 |
| EMPASC-102 | - | 7.29 | 8.82 | 4.82 | 6.68 | 8.60 | 7.56 | 8.10 | 5.64 | 6.68 | 8.06 | 7.23 |
| EMPASC-101 | - | 8.74 | 9.54 | 5.69 | 7.44 | 6.71 | 7.69 | 7.72 | 5.65 | 6.51 | 8.45 | 7.41 |
| BR-IRGA-409 | - | 6.56 | 7.34 | 6.50 | 6.05 | 6.70 | - | 7.50 | 5.26 | 4.32 | 7.31 | 6.39 |
| BR-IRGA-410 | - | 6.94 | 8.18 | 5.43 | 6.03 | 6.20 | - | 7.72 | 5.67 | 4.09 | 7.54 | 6.42 |
| IR 841 | - | 6.51 | 9.21 | 8.69 | 8.16 | 10.98 | 7.88 | 6.59 | 4.05 | 6.45 | 7.71 | 7.62 |
| Mean | | 7.22 | 8.10 | 6.41 | 6.67 | 7.36 | 7.01 | 6.81 | 4.98 | 5.91 | 7.89 | 6.84 |

A number of mutants were selected from populations of irradiated Pratão Precoce in respect to plant height, grain type, and lodging resistance but all were discarded because of instability as well as their very low tiller number. No mutants were identified in the $M_2$ generation derived from irradiated seeds of CICA 8.

TABLE IV. MEAN MILLING YIELD, CHALKINESS AND OTHER AGRONOMIC TRAITS OF IRRIGATED RICE ADVANCED MUTANT LINES AT FIVE LOCATIONS IN SANTA CATARINA STATE, EMPASC/EEI, 1990/91.

| Line or cultivar | Milling yield (%) | | Chalkiness (0-5) | Plant height (cm) | Blight | | Iron toxicity | Lodging (0-9) | Life cycle (days) |
|---|---|---|---|---|---|---|---|---|---|
| | Whole grain | Total grain | | | Leaf (0-9) | Neck (0-9) | | | |
| SC-97 | 63.7 | 71.0 | 2 | 93 | 2.0 | 2.3 | 5 | 5 | 107 |
| SC-110* | 66.6 | 69.6 | 2 | 86 | 3.7 | 5.7 | 7 | 3 | 102 |
| SC-100** | 58.4 | 69.8 | 1 | 94 | 2.3 | 1.0 | 4 | 1 | 104 |
| BR-IRGA-414 | 67.2 | 70.3 | 2 | 90 | 4.0 | 7.7 | 4 | 5 | 101 |
| SC-99 SC-99 | 56.7 | 68.9 | 1 | 93 | 3.0 | 3.7 | 7 | 3 | 105 |
| SC-111* | 64.2 | 68.9 | 2 | 93 | 3.7 | 5.0 | 7 | 9 | 105 |
| SC-71 | 68.2 | 71.5 | 2 | 81 | 3.0 | 6.3 | 7 | - | 100 |
| SC-96 | 63.0 | 69.3 | 2 | 91 | 2.0 | 1.7 | 6 | 9 | 109 |
| SC-104 | 61.4 | 68.5 | 1 | 95 | 2.7 | 1.7 | 4 | 7 | 109 |
| SC-105 | 68.4 | 71.3 | 1 | 81 | 3.7 | 7.0 | 4 | - | 110 |
| SC-101 | 63.8 | 69.6 | 1 | 92 | 3.0 | 3.0 | 7 | 3 | 111 |
| SC-117** | 64.6 | 70.4 | 2 | 87 | 3.0 | 3.7 | 6 | 3 | 120 |
| EMPASC-105 | 63.7 | 71.1 | 2 | 93 | 2.7 | 2.0 | 7 | - | 130 |
| SC-24* | 68.9 | 71.3 | 1 | 92 | 2.7 | 2.3 | 4 | - | 123 |
| SC-2** | 65.1 | 71.1 | 3 | 85 | 4.0 | 5.0 | 4 | 3 | 141 |
| CICA 8 | 65.8 | 72.0 | 3 | 85 | 3.0 | 5.0 | 4 | 7 | 142 |
| EMPASC-104 | 58.5 | 70.0 | 3 | 88 | 2.3 | 3.7 | - | - | 126 |
| EMPASC-102 | 64.8 | 72.3 | 4 | 95 | 3.3 | 1.0 | 5 | 5 | 128 |
| EMPASC-101 | 64.5 | 71.7 | 4 | 96 | 2.3 | 1.0 | 5 | 5 | 128 |
| BR-IRGA-409 | 64.3 | 68.4 | 2 | 104 | 3.7 | 3.0 | 7 | 9 | 108 |
| BR-IRGA-410 | 59.2 | 64.6 | 3 | 95 | 4.0 | 7.7 | 5 | 3 | 109 |
| IR-841 | 63.1 | 70.3 | 3 | 83 | 4.3 | 3.0 | 4 | - | 138 |

* Discarded.

** Advanced to regional trials.

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# RADIOBIOLOGICAL EFFECTS OF RADIATION OF DIFFERENT LETs ON CUBAN RICE CULTIVAR J-104

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**Abstract**

The effects of radiations of different LET's were assessed by their inhibition of root growth 14 days after sowing. The radiations used were neutrons and gamma. The data were adjusted using the polynomial $Er = 1-(1-e^{kD})^4$, where Er is the mean for root length at different doses. The doses giving growth reduction values of 50, 30, 20, and 10% were determined. The calculated RBE values were for $^{137}Cs$--1, neutrons--12.7, protons--4.4, bremstrahlung--1.6, $\pi$--30.5. Also, the sensitivity values, k, were calculated for each radiation.

## 1. INTRODUCTION

Various authors have reported the effects of radiation of different LET's on microorganisms, tissue cultures, and plants [1-5]. In rice, the effects of gamma rays and neutrons have been reported [4]. Deus *et al.* [6] obtained mutants of different types when the rice cultivar J-104 was irradiated with gamma rays ($^{60}Co$) and 14 Mev fast neutrons. In the literature, we have not found any data on the effects of protons, n mesons, or bremstrahlung of 13 Mev on rice cultivars.

The present work in the Cuban rice breeding programme was carried out to study the effects of radiations of different LETs on the Cuban rice cultivar J-104.

## 2. MATERIALS AND METHODS

One hundred dry seeds per radiation dose were exposed to 1.5 Mev neutrons in the biological channel of the IBR-2 pulsed reactor facilities with a gamma rays contamination of less than 5% with a dose rate of 0.36 Gy/min, and to gamma rays of $^{137}Cs$ with a dose rate of 4.8 Gy/min. At the Phasotron facilities, the seeds were irradiated with 160 Mev protons at a dose rate of 3 Gy/min, and n-mesons of 40 Mev at a rate of 4.8 Gy/hr. The register of protons and n-mesons was conducted with a dosimeter type VA-J-18, with a spherical ionizing chamber type VA-k-253 [7,8] (Laboratory of Nuclear Problems). Bremstrahlung (13 Mev) was obtained at the microtran facilities (model MT-25) calibrated with a LiF dosimeter and pure gold liners. The rice cultivar used in this study was J-104. The moisture content of the seed was 11.5%.

The treated seeds were sown in controlled conditions of humidity, temperature, and light, and the root length of the seedlings was measured 14 days after sowing [9]. The Tonystat and Brasier statistical programs were used to analyze the data on an IBM microcomputer. The confidence levels were 95-98%.

The dose response curve can be approximated by the following function:

$$Er = 1 - (1 - e^{kD})^4$$

$$\text{where } Er = \frac{Ex - En}{Eo - En}$$

Ex = mean value of the root length at x doses
En = mean value of the root length at the latest dose
Eo = mean value of the root length of the control [10]

This work was carried out at the irradiation facilities of JINR Laboratory of Neutron Physics, Dubna, Russia.

## 3. RESULTS AND DISCUSSION

The effects of gamma rays, neutrons, protons, n mesons, and bremstrahlung (13 Mev) are shown in Figures 1 and 2. The effects of gamma radiation on root length has been described by Ukai [10]. The results we obtained can be fitted by the same function reported by the cited author, not only for gamma rays but also for the other types of radiation employed, in the way we analyzed the results for n mesons, protons, and neutrons. As can be seen, the way the LET changes the profile of the curves is more acute. This agrees with the findings with *Tradescantia* and *Vicia faba* [11-13], when x-rays, neutrons of different energies, 25 and 250 kev photons, $^{60}Co$ gamma rays, and 18 Mev electrons were used.

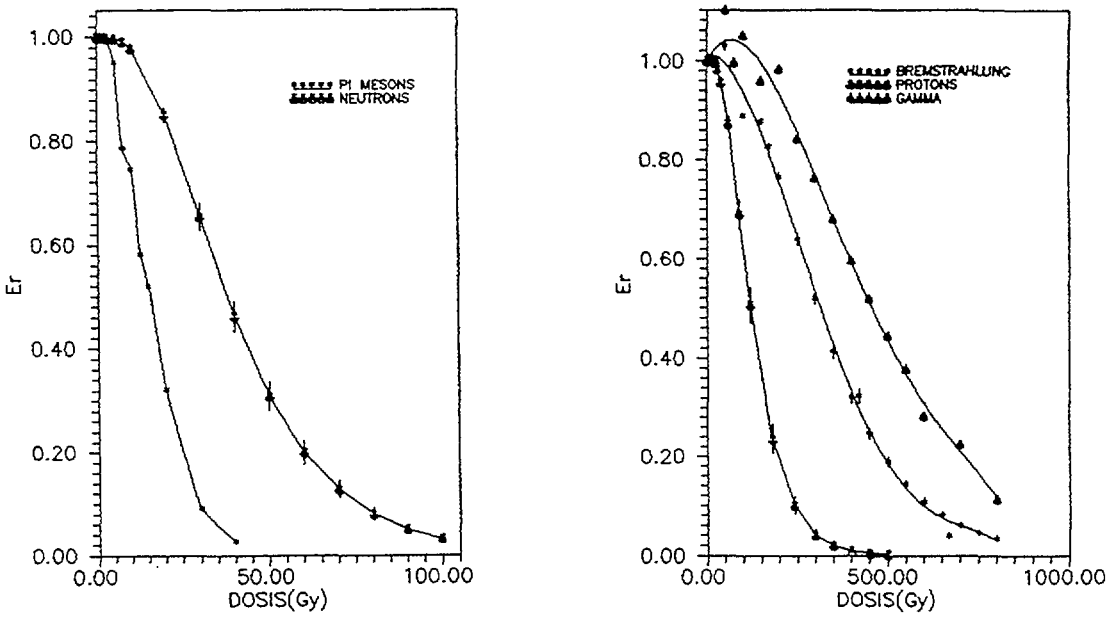


*FIG. 1. Root length of Cuban rice cultivar J104 as affected by: A. neutrons and π mesons, B. gamma, bremstrahlung and proton irradiations.*

Table I presents the radiobiological values calculated from the function described by Ukai [10]. Sharma [14] reported $LD_{50}$ ranging from 250 to 400 Gy gamma rays for different *indica* and *japonica* rice cultivars. Other authors reported an $LD_{50}$ of 250 Gy for the Hungarian rice variety Dungbam Shali [15]. These authors also reported an $LD_{50}$ value of 27 Gy for fast neutrons. The RBE found for gamma ray/neutron was about 10. For the Cuban variety J-104, a growth reduction of 50% was obtained with a dose of 464 Gy for gamma rays and 39.06 Gy for fast neutrons. For other rice varieties grown in Cuba, like Caribe-1 and IR-1529-30, the 50% growth reduction dose was 697 and 580, respectively, when dormant seeds were irradiated with gamma rays [16]. The RBE for gamma /neutron was 12.7. For n mesons, protons, and bremstrahlung, the 50% growth reduction values were 15.38, 128.04, and 321.55 Gy, respectively. The RBE values for these radiations were 30.52, 4.4, and 1.6. Maslov [17] reported that the effects of protons on wheat seeds are between those caused by neutrons and gamma rays. The k value for every radiation is given in Table I. As is seen, as the effects are more acute, the value increases. This agrees with the values given for Do in [18] when radiations with different LETs were used with yeast.

TABLE I. EFFECTS OF NEUTRONS AND Π-MESSONS ON ROOT LENGTH OF CUBAN RICE CULTIVAR J104.

| Parameter | Π-Mesons | $\gamma(^{137}Cs)$ | Protons | Bremstrahlung | Neutrons |
|---|---|---|---|---|---|
| $Gr_{50}$(Gy) | 15.38 | 463.99 | 128.04 | 321.55 | 36.06 |
| $Gr_{30}$(Gy) | 11.01 | 358.83 | 94.27 | 228.28 | 29.23 |
| $Gr_{20}$(Gy) | 8.54 | 299.39 | 75.23 | 175.21 | 23.69 |
| $Gr_{10}$(Gy) | 5.52 | 226.27 | 51.94 | 111.38 | 16.90 |
| RBE | 30.52 | 1.00 | 4.40 | 1.60 | 12.70 |
| $K(Gy^{-1})$ | 0.070 | 0.0023 | 0.010 | 0.00369 | 0.293 |

## ACKNOWLEDGEMENTS

We appreciate the use of the facilities at JINR, Dubna, Russia, and the help extended by the head of the Activation Analysis Group, V. M. Nazarov (Laboratory of Neutron Physics), and by the staff of the Phasotron facilities, V. Abaso and A. Cherevatenko. We also wish to thank A. Belov and Teterov Yu at the microtom facilities for their kindness and fruitful advice during the course of the experiments.

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# RICE IMPROVEMENT THROUGH INDUCTION OF MUTATIONS WITH GAMMA RAYS

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**Abstract**

The objective of this research was to induce mutations for early maturity, resistance or tolerance to blast (*Pyricularia grisea*), and high yield potential in the rice (*O. sativa*) varieties ICTA Virginia and Precozicta. Six thousand seeds of each variety were irradiated and the $M_1$ generation was grown. The irradiation doses for Precozicta and ICTA Virginia were 31 and 29 krad (310 and 290 Gy), respectively. One row from each plant selected in the $M_1$ generation was sown in the field. Mutants that possessed high yield potential, early maturity, resistance to blast, chlorophyll deficiencies, and morphological differences were identified and selected. The $M_3$ generation of mutants identified in the $M_2$ generation was sown in the field to confirm the $M_2$ classifications. Early maturing and blast resistant mutants were harvested. Forty eight mutant lines selected in the $M_3$ generation were evaluated in replicated field plots in the $M_4$ generation.

## 1. INTRODUCTION

Rice is one of the most important crops in the world. In Guatemala, corn, common beans, potatoes and rice constitute the daily diet of the population. The two most important varieties in Guatemala are ICTA Virginia and Precozicta. They are semidwarf varieties with superior yielding ability but they have become susceptible to the blast disease, caused by *Pyricularia grisea*, the most serious disease of rice in Guatemala and the major constraint to higher yields. A mutation breeding program was initiated in 1986 with the objectives of developing blast resistant and earlier maturing lines of ICTA Virginia and Precozicta.

## 2. MATERIALS AND METHODS

### *Genetic material*

ICTA Virginia and Precozicta were introduced from CIAT. The pedigree of ICTA Virginia is P918-25-1-4-2-3-1B/3/CICA4//$F_1$ IR665-23-3/Tetep and that of Precozicta is P790-B4-4-1T//IR9302/IR665-31-2-4 [1].

### *Radiation treatments*

Seeds were irradiated with 0, 15, 20, 25, 30 and 40 krad (0, 150, 200, 250, 300 and 400 Gy). The irradiated seeds were sown in the greenhouse and field to establish the radiation sensitivity dose for each variety [2].

*The $M_1$ generation*

Based on the sensitivity data, 6000 seeds of each variety were irradiated. The plants were inspected to identify effects of somatic mutations [3]. Two panicles of each surviving plant were harvested and maintained separately [2,4].

*The $M_2$ generation*

Twenty seeds from each panicle harvested in the $M_1$ generation were sown in separate plots in the $M_2$ generation. The plants were inspected for mutations. Seeds from all mutants were harvested on an individual plant basis. In addition, plants that showed no morphological differences and control plants were harvested for evaluation in the following generation.

*The $M_3$ generation*

Seeds from mutant, apparently normal, and control plants harvested in the $M_2$ generation were sown in field plots. Blast susceptible varieties were sown in the experimental area to serve as sources of *Pyricularia grisea* inoculum. All mutant populations were inspected to confirm their genetic stability as well as to evaluate their agronomic characteristics and blast reaction. The reaction to blast was based on the standard evaluation scale for rice [5].

*The $M_4$ generation*

Forty eight lines from each variety were evaluated in field plots of 6 rows x 5 meters in length, in two replications, to evaluate their agronomic characteristics. They included lines classified as mutants in the $M_3$ generation as well as tall lines with morphological changes superior to the original varieties.

*The $M_5$ generation*

Ten mutants from the $M_4$ generation were evaluated in the $M_5$ generation. A complete randomized block design with three replications was used for this evaluation. Each mutant was sown in a plot of 6 rows x 5 meters in length with 0.30 m between rows.

## 3. RESULTS AND DISCUSSION

Somatic effects were observed in the $M_1$ generation. Reduction in number of surviving plants and in plant height of ICTA Virginia are presented in Figures 1 and 2. Radiation dose ranges of 27 to 34 krads showed a 28% reduction in germination and a 30% reduction in plant height in the greenhouse. In the field, the same dose range resulted in a 30% reduction in plant survival and a 17% reduction in plant height. These results are in agreement with the report of Escuro and Guevarra [6].

For Precozicta, the dose range of 30 to 35 krad showed a 30% reduction in germination (Fig. 3) and 30% reduction in plant height in the greenhouse. In the field, plant survival was reduced by 30% and plant height by 15%. Similar results were reported by Thann and Trinh [7].

In the $M_2$ generation, with the doses of 29 and 31 krads for ICTA Virginia and Precozicta, respectively, putative mutants were selected from a population of 48 000 plants. They included mutations for morphological characters, chlorophyll deficiencies, earlier maturity, resistance to blast, and higher yield potential. Several plants selected in $M_2$ were confirmed as mutants in $M_3$. Table I

includes the most significant mutants obtained in the $M_3$. Among these mutants was one that was 15 days earlier than the parent variety, ICTA Virginia. From Precozicta, one mutant was 10 days and a second mutant 20 days earlier than the original variety. Blast resistance mutants were also confirmed in the $M_3$ for ICTA Virginia (Table II) and Precozicta (Table III).

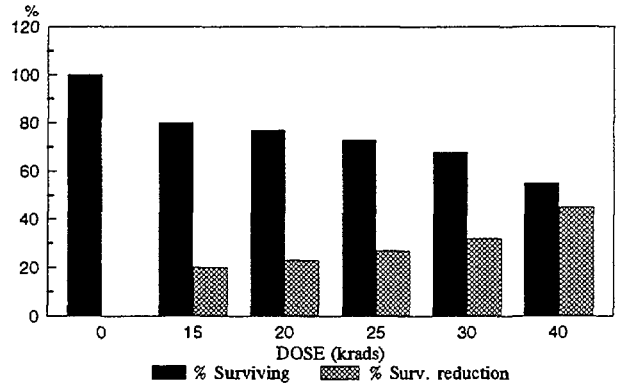


*FIG. 1. $M_1$ survival following gamma rays treatments of ICTA Virginia seeds.*

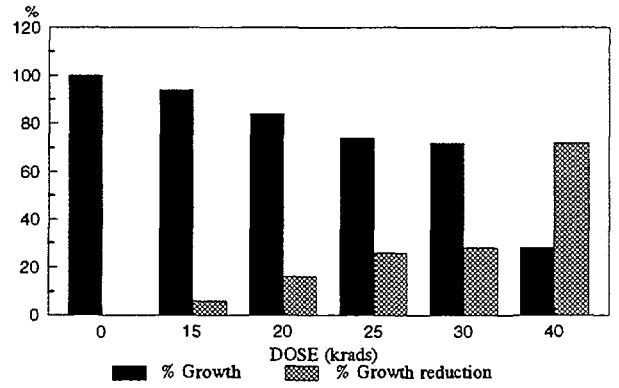


*FIG. 2. $M_1$ growth reduction following gamma rays treatments of ICTA Virginia seeds.*

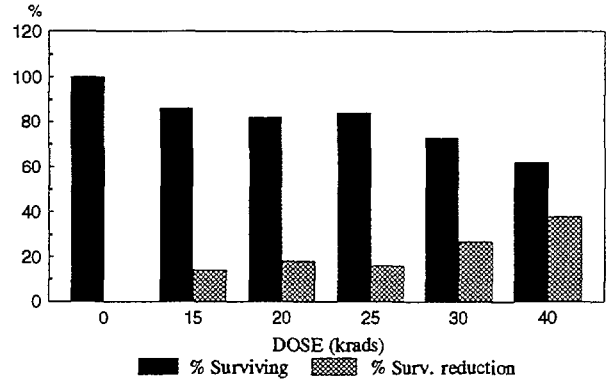


*FIG. 3. $M_1$ survival following gamma rays treatments of Precozicta variety seeds.*

TABLE I. MUTANTS CONFIRMED IN THE $M_3$ GENERATION.

| Variety | Number | Mutant characters |
|---|---|---|
| ICTA Virginia | 5 | Tolerance to *Pyricularia* (a 1 rating) |
| " | 1 | 15 days earlier than control |
| " | 2 | Morphological differences |
| " | 2 | Chlorophyll deficiencies |
| Precozicta | 1 | Tolerance to *Pyricularia* (an 0 rating) |
| " | 1 | Tolerance to *Pyricularia* (a 1 rating) |
| " | 2 | 10 and 20 days earlier than control |

TABLE II. DISTRIBUTION FOR REACTION TO BLAST (% OF POPULATION) OF 1032 LINES FROM ICTA VIRGINIA.

| Blast reaction* | Mutants and putative mutants | | Control | |
|---|---|---|---|---|
| | Leaf (%) | Neck (%) | Leaf (%) | Neck (%) |
| 0 | - | - | - | - |
| 1 | 0.58 | 0.49 | - | - |
| 3 | 5.53 | 3.79 | 17.95 | 3.25 |
| 5 | 32.62 | 24.32 | 42.31 | 25.20 |
| 7 | 60.80 | 41.44 | 39.74 | 39.03 |
| 9 | 0.50 | 29.96 | - | 32.52 |

* 0 = no blast and 9 = severe blast.

TABLE III. DISTRIBUTION FOR REACTION TO BLAST(% OF POPULATION) OF 1068 LINES FROM PRECOZICTA.

| Blast reaction* | Mutants and putative mutants | | Control | |
|---|---|---|---|---|
| | Leaf (%) | Neck (%) | Leaf (%) | Neck (%) |
| 0 | - | 0.095 | - | - |
| 1 | 0.09 | 0.10 | - | - |
| 3 | 2.53 | 9.10 | 7.36 | 0.89 |
| 5 | 27.77 | 32.40 | 33.82 | 37.52 |
| 7 | 69.61 | 56.20 | 58.82 | 59.80 |
| 9 | - | 1.30 | - | 1.79 |

* 0 = no blast and 9 = severe blast.

More than forty lines from each variety were evaluated in the $M_4$ generation for the following agronomic characteristics: days to flowering, height, leaf blast, neck blast, leaf scald, brown spot and yield. Among ICTA Virginia lines, significant differences were found for days to flowering, neck blast and yield. Among Precozicta lines, significant differences were observed for days to flowering, height, leaf blast, neck blast and yield.

Five mutant lines derived from ICTA Virginia combine blast resistance and higher yielding ability than the parent variety (Lines 620, 881, 940, 568 and 1001). From Precozicta also, 5 lines were identified that have superior blast resistance and higher yield potential than the parent variety (Lines 1796, 2032, 1146, 2097 and 1736). The blast reaction and agronomic characteristics of these 10 superior mutants are presented in Table IV.

TABLE IV. AGRONOMIC CHARACTERISTICS AND DISEASE REACTIONS OF THE MOST PROMISING $M_4$ MUTANTS AND CONTROLS.*

| Mutant | Days to heading | Height (cm) | Blast | | Leaf scald | Brown spot | Yield (kg/ha) |
|---|---|---|---|---|---|---|---|
| | | | Leaf | Neck | | | |
| V620 | 100 | 106 | 1 | 0 | 6 | 1 | 7816 |
| V881 | 100 | 100 | 1 | 1 | 6 | 0 | 7284 |
| V940 | 100 | 103 | 1 | 2 | 7 | 1 | 7227 |
| V568 | 100 | 104 | 1 | 3 | 4 | 1 | 6932 |
| V1001 | 100 | 94 | 1 | 4 | 5 | 1 | 6826 |
| ICTA Virginia | 100 | 98 | 1 | 4 | 6 | 1 | 6743 |
| P1796 | 100 | 100 | 1 | 0 | 6 | 0 | 6183 |
| P2032 | 96 | 108 | 1 | 2 | 4 | 1 | 8225 |
| P1146 | 88 | 108 | 3 | 3 | 6 | 1 | 6693 |
| P2097 | 98 | 96 | 1 | 4 | 4 | 1 | 6075 |
| P1736 | 100 | 100 | 2 | 4 | 4 | 0 | 5838 |
| Precozicta | 90 | 101 | 6 | 7 | 6 | 1 | 4991 |

* For disease ratings, 0 = no disease and 9 = severe disease.

In the evaluation of the $M_5$ generation (10 lines selected in the $M_4$), for the incidence of leaf blast (Bl), neck blast (NBl) and leaf scald (LSc) was important. The evaluations are presented in Table V.

The mutants derived from ICTA Virginia showed resistance to leaf blast, with ratings of 1 and 2 on the scale. The mutants MV-881, MV-940 and MV-1001, maintained their resistance to neck blast (rating of 1). The control was moderately susceptible.

The lines MPI-1736, MPI-2032, and MPI-2097, derived from Precozicta, showed resistance to leaf blast. The control was moderately susceptible. For neck blast, all mutants were better than the control (rating of 7), but the best was MPI-1736 with moderate resistance (a 3 rating). Concerning leaf scald, caused by *Rhynchosporium oryzae*, all the lines exhibited moderate resistance to moderate susceptibility, the same as the controls.

TABLE V. AGRONOMIC CHARACTERISTICS OF THE $M_5$ MUTANT LINES.

| Genotype | Vg | Fl | Sen | Lg | Exs | Ht | PAcp | Bl | NBl | LSc | Bs | Yld |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| ICTA Virginia | 3 | 96 | 3 | 2 | 1 | 91 | 4 | 1 | 4 | 2 | 1 | 7.37 |
| MV-881 | 3 | 96 | 1 | 1 | 1 | 95 | 3 | 1 | 1 | 4 | 1 | 7.32 |
| MV-1001 | 4 | 94 | 4 | 2 | 1 | 91 | 3 | 1 | 2 | 3 | 1 | 7.15 |
| MV-568 | 4 | 95 | 4 | 1 | 2 | 93 | 4 | 2 | 5 | 4 | 1 | 6.67 |
| MV-620 | 3 | 92 | 1 | 1 | 1 | 100 | 3 | 2 | 3 | 4 | 1 | 6.60 |
| MPI-1736 | 4 | 91 | 4 | 3 | 2 | 94 | 5 | 2 | 3 | 3 | 1 | 6.45 |
| MV-940 | 4 | 93 | 1 | 1 | 1 | 97 | 3 | 1 | 1 | 4 | 1 | 6.17 |
| MPI-2032 | 3 | 90 | 2 | 2 | 3 | 99 | 5 | 2 | 5 | 3 | 1 | 6.10 |
| MPI-2097 | 3 | 90 | 2 | 1 | 3 | 97 | 5 | 1 | 5 | 3 | 1 | 6.08 |
| MPI-1146 | 3 | 75 | 4 | 1 | 4 | 95 | 5 | 4 | 5 | 3 | 1 | 5.01 |
| MPI-1796 | 5 | 93 | 3 | 1 | 4 | 97 | 5 | 4 | 5 | 4 | 1 | 4.56 |
| Precozicta | 3 | 81 | 4 | 2 | 3 | 100 | 3 | 4 | 7 | 3 | 1 | 4.46 |

Vg = Vigor; Fl = Days to flowering; Sen = Senescence; Lg = Lodging; Exs = Exsertion of panicle; Ht = Height; PAcp = Phenotype acceptability; Bl = Leaf blast; NBl = Neck blast; LSc = Leaf scald; Bs = Brown spot; Yld = Yield (t/ha)

## 4. CONCLUSIONS

The optimum radiation dose for ICTA Virginia and Precozicta was 29 and 31 krad, respectively. From 48 000 plants in the $M_2$ generation, mutants were identified in respect to differences in morphological characteristics, chlorophyll deficiencies, maturity, yield potential, and reaction to blast. The results showed that in many mutants, blast tolerance was induced. In the $M_4$ generation, 48 lines were evaluated and, based on statistical analyses, 5 mutants were selected from each variety because of higher grain yields and better tolerance or resistance to blast than the original varieties. The average grain yield of ICTA Virginia and Precozicta mutants was 7217 and 6603 kg/ha, respectively. The grain yield for the original varieties ICTA Virginia and Precozicta was 6743 and 4991 kg/ha, respectively. All of the early maturing mutants were susceptible to blast. The blast ratings for the five mutants selected in the $M_4$ of Precozicta were in the range of 1- 3 and 1-4 for leaf blast and neck blast, respectively, while the original variety was in the range of 6-7 for both traits.

Five of the lines evaluated in the $M_5$ generation gave resistant to moderately resistant reactions to blast, caused by *Pyricularia grisea*, and the others were moderately susceptible. Considering the fact that the parental varieties are highly susceptible to this pathogen, we can conclude that a significant level of resistance was developed in the selected mutants.

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# MUTATION BREEDING OF RICE IN COSTA RICA

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**Abstract**

Seeds of rice (*Oryza sativa L.*) cultivar CR-1113, which is susceptible to the blast disease (*Pyricularia grisea*), were subjected to gamma irradiation with $^{60}Co$, using doses of 15 and 20 krad (150-200 Gy) to select mutants with resistance or partial resistance to the disease. A total of 80 000 plants per treatment were individually evaluated in the $M_2$ generation at the tillering and panicle emergence stages. Partial resistance was observed in 45 plants from the 15 krad and 8 plants from the 20 krad treatment. From 52 harvested lines, 7 were selected for their good agronomic performance, good yield, and partial resistance to blast and 7 for their earliness and good agronomic traits. The selected lines were evaluated in field trials. Results confirmed their partial resistance to blast and good agronomic characteristics.

## 1. INTRODUCTION

Rice (*Oryza sativa L.*) is a basic food of the Costa Rican people. It is a very important crop from both a production and a consumption standpoint. Approximately 65 000 ha of rice are grown annually, and the average yield is 3.12 t/ha.

Most of the rice in Costa Rica is cultivated in relatively level fields with a slight slope. It is grown predominantly as an upland crop and depends on rainfall for moisture, but the irrigated acreage is increasing. The rice-growing areas of the country are characterized by high temperatures and high humidity, an environment that fosters the growth of fungal disease microorganisms. Among them, *Pyricularia grisea*, the blast disease pathogen, is one of the most important, reducing yield as much as 90% in severe attacks.

The main objective of this project was to induce genetic variability in the principal rice cultivars in Costa Rica by irradiating seed with $^{60}Co$ gamma rays in order to select plants resistant or tolerant to the blast disease in combination with good agronomic characteristics and grain quality.

## 2. MATERIALS AND METHODS

*First phase*

To establish the radiosensitivity of the principal rice cultivars in Costa Rica, seeds of CR-1113, Cr-5272, and CR-201 with a moisture content of 12% were exposed to $^{60}Co$ gamma rays, using doses

of 10, 20, 30, 40, and 50 krad. The "sandwich" or "growing rack" method [1] described by Mikaelsen (1966) and mentioned by IAEA (1977), was used. A complete randomized experimental design with four replications was used. Seeds were placed in a growth chamber with alternating temperatures of 20/30°C (8 and 16 hours, respectively) and lights on during the 30°C period.

Features measured included germination rate (GR), emergence decrease index (EDI), growth decrease index (GDI), and seedling height. Measurements commenced at the beginning of the germination period and were made every two days for two weeks.

*Second phase*

Seeds of CR-1113, developed by the National Rice Program (Pedigree IR822-81-CR-2), were irradiated with 15 and 20 krad to obtain seeds for the $M_1$ generation, which was sown in a pathogen-free field. The $M_1$ was harvested and the $M_2$ generation was sown in a field with high inoculum pressure. A total of 80 000 plants per treatment were individually evaluated at growth stages 2 and 4, according to the criteria of the IRRI-CIAT programme [2]. From the $M_2$ population, 53 plants were identified as possibly being resistant to blast.

*Third phase*

Seeds of the 53 plants selected in the $M_2$ were sown in nursery beds to verify their blast reaction. One of the plants produced only albino seedlings, therefore the final number of putative resistant lines was 52. Fifteen-day old seedlings of the 52 lines were transplanted in a field with a high inoculum pressure. The plot size for each line was 4 rows X 50 m, with 0.30 m between rows. As a control and a secondary inoculum source, 2 rows of CR-1113 were planted between the plots.

The evaluation for blast resistance was conducted at growth stages 2 and 7, according to the IRRI-CIAT scale. Seven lines were selected, based on their good agronomic performance, good yield, and partial resistance to blast, and an additional 7 lines were selected because of their early maturity relative to the parent cultivar.

*Fourth phase*

Two seed multiplication cycles of the selected mutant lines were carried out in a farmer's field in 1990 to provide sufficient seed for further testing. Two yield trials were then conducted. One trial included the seven mutant lines selected for improved blast tolerance, good general performance, and good yield. The second trial included the seven early-maturing mutant lines. Both tests used a randomized block design with four replications and the cultivars CR-1113, CR-5272, and CR-1821 as checks. The size of individual plots was 3.5 X 2.5 m (8.75 $m^2$). The distance between rows within a plot was 0.2 m. An area 6 $m^2$ was harvested to determine grain yield.

The following traits were measured in the yield trials: number of days to flowering, flag leaf length, lower leaf (penultimate leaf) length, plant height, panicle length, 1000-grain weight at 12% moisture, number of filled grains per panicle, number of empty spikelets per panicle, estimated yield in t/ha, and response to blast at the seedling, preheading, and grain-filling growth stages.

The mutant lines selected for improved tolerance to blast and good agronomic performance were UN-9001,UN-9002, UN-9017, UN-9026, UN-9027, UN-9038, and UN-9051. The early-maturing mutant lines were UN-9006, UN-9008, UN-9012, UN-9014, UN-9025, UN-9039 and UN-9040.

## 3. RESULTS AND DISCUSSION

*First phase*

Table 1 presents the data for the germination rate, emergence decrease index, and growth decrease index. With the cultivars CR-1113 and CR-201, GR increased slightly relative to the control at 10 and 20 krad, with CR-5272 showing no effect at those rates. At 30 krad, GR for all cultivars decreased moderately, and at 40 krad all exhibited a severe reduction in GR. A radiation dose of 50 krad was lethal for all cultivars.

TABLE I. EMERGENCE DECREASE INDEX (EDI), GROWTH DECREASE INDEX (GDI) AND GERMINATION RATE (GR) IN THREE VARIETIES TREATED WITH GAMMA RAYS.

| | CR-1113 | | | CR-5272 | | | CR-201 | | |
|---|---|---|---|---|---|---|---|---|---|
| Dose (krad) | GR(%) | EDI | GDI | GR(%) | EDI | GDI | GR(%) | EDI | GDI |
| 0 | 0.75 | 0.00 | 0.00 | 81.00 | 0.00 | 0.00 | 89.50 | 0.00 | 0.00 |
| 10 | 91.75 | -3.38 | 7.50 | 82.75 | -2.16 | 1.96 | 89.75 | -0.28 | -0.77 |
| 20 | 9.75 | -5.63 | 15.04 | 78.50 | 3.09 | 8.46 | 90.50 | -1.12 | 20.75 |
| 30 | 82.75 | 6.67 | 49.66 | 74.00 | 8.64 | 32.63 | 83.50 | 6.71 | 50.67 |
| 40 | 36.25 | 59.13 | 80.79 | 28.75 | 64.51 | 68.55 | 47.00 | 47.50 | 79.31 |

EDI was negative at doses of 10 and 20 krad for CR-1113 and CR-201 and at 10 krad for CR-5272, indicating increased seedling emergence relative to the control at those dosages. At an irradiation level of 30 krad the EDI for the three cultivars showed a moderate positive increase, and at 40 krad, a strong positive increase. GDI intensified as dosage increased, reaching very marked levels at 40 krad. Seedling height of the three cultivars was negatively affected at dosages above 10 krad (Table II).

*Second phase*

From the $M_2$ population that received the 15 krad dose, 48 plants were identified as possible mutants with increased tolerance to blast. At the 20 krad dose, only 6 possible mutants were selected. All of the putative mutants were evaluated at growth stages 2 and 4 under field conditions with high inoculum pressure, using the IRRI-CIAT scale. The agronomic traits of the selected mutants and the control are presented in Table III and IV. The frequency of mutant plants was $1.37 \times 10^{-3}$ for 15 krad and $2 \times 10^{-4}$ for 20 krad. The frequencies were calculated according to the following formula:

$$\text{Frequency of mutant plants} = \frac{\text{number of mutant plants}}{\text{number of total plants}}$$

*Third phase*

Table V presents agronomic data and blast reaction for 52 mutant lines in the $M_3$ generation. Based on the IRRI-CIAT scale, 37 of the lines were moderately resistant to blast. Twenty-two of the lines contained albino plants, varying from 1 to 40% of the plants within a line. Although the main goal of the project was to obtain mutants with improved tolerance to blast, seven early maturing

mutants were also selected. Table VI presents the means and the ranges for the agronomic and morphological traits of the mutants in the $M_3$ generation.

TABLE II. MEAN VALUES OF $M_1$ PLANTLETS HEIGHT OF THREE VARIETIES OF RICE.

| Dose (krad) | CR-1113 | | CR-5272 | | CR-201 | |
|---|---|---|---|---|---|---|
| | Height (mm) | Percent | Height (mm) | Percent | Height (mm) | Percent |
| CONTROL | 124.94 a | 100.00 | 83.76 a | 100.00 | 101.01 a | 100.00 |
| 10 | 115.57 b | 92.50 | 83.40 ab | 102.00 | 101.79 a | 101.80 |
| 20 | 106.15 c | 84.96 | 76.67 b | 91.54 | 80.06 b | 79.26 |
| 30 | 62.90 d | 50.34 | 56.43 c | 67.37 | 49.33 c | 49.33 |
| 40 | 24.00 e | 19.21 | 26.34 d | 31.45 | 20.90 d | 20.69 |
| 50 | -- | -- | -- | -- | -- | -- |

* Values followed by the same letter are not statistically different at the 5% level, according to the Duncan Test (5%).

TABLE III. CHARACTERISTICS OF THE SELECTED $M_2$ PLANTS IN RELATION TO THE CONTROL (CR-1113).

| Selection No. | No. of tillers and panicles per plant | Days to harvest No. | Yield per plant (g) | Blast response (0-9) |
|---|---|---|---|---|
| CONTROL* | 27 | 134 | 13.88 | 9 |
| 15 krad | | | | |
| 1 | 25 | 177 | 30.33 | 1 |
| 2 | 25 | 112 | 15.89 | 2 |
| 3 | 24 | 127 | 33.93 | 1 |
| 4 | 50 | 127 | 45.46 | 1 |
| 5 | 23 | 127 | 24.75 | 1 |
| 6 | 29 | 127 | 33.81 | 1 |
| 7 | 39 | 127 | 29.99 | 3 |
| 8 | 45 | 127 | 25.95 | 1 |
| 9 | 16 | 127 | 14.71 | 2 |
| 10 | 12 | 127 | 17.17 | 2 |
| 11 | 21 | 127 | 31.60 | 3 |
| 12 | 17 | 127 | 19.14 | 3 |
| 13 | 25 | 127 | 23.61 | 2 |
| 14 | 16 | 127 | 11.14 | 3 |
| 15 | 26 | 127 | 32.99 | 3 |
| 16 | 17 | 127 | 14.77 | 1 |
| 17 | -- | -- | -- | - |
| 18 | 20 | 127 | 11.43 | 1 |
| 19 | 30 | 127 | 28.62 | 1 |
| 20 | 37 | 127 | 50.00 | 1 |
| 21 | 20 | 127 | 24.59 | 1 |
| 22 | 14 | 127 | 20.43 | 2 |
| 23 | 18 | 127 | 21.66 | 2 |
| 24 | 20 | 127 | 23.07 | 2 |
| 25 | 20 | 127 | 26.67 | 1 |
| 26 | 14 | 127 | 6.35 | 2 |

TABLE III. (Cont.)

| Selection No. | No. of tillers and panicles per plant | Days to harvest No. | Yield per plant (g) | Blast response (0-9) |
|---|---|---|---|---|
| 27 | 20 | 127 | 24.52 | 1 |
| 28 | 25 | 127 | 37.94 | 1 |
| 29 | 20 | 127 | 13.46 | 1 |
| 30 | 13 | 127 | 11.21 | 1 |
| 31 | 14 | 127 | 24.23 | 1 |
| 32 | 20 | 127 | 9.13 | 2 |
| 33 | 37 | 127 | 27.25 | 1 |
| 34 | 18 | 127 | 37.97 | 1 |
| 35 | 27 | 127 | 15.89 | 1 |
| 36 | 14 | 127 | 17.01 | 1 |
| 37 | 31 | 127 | 43.65 | 2 |
| 38 | 20 | 127 | 12.85 | 1 |
| 39 | 15 | 127 | 20.56 | 1 |
| 40 | 25 | 127 | -- | 2 |
| 41 | 31 | 127 | 45.37 | 1 |
| 42 | 24 | 127 | 30.47 | 1 |
| 43 | 49 | 127 | 62.99 | 1 |
| 44 | 18 | 127 | 23.27 | 1 |
| 45 | 17 | 127 | 23.61 | 1 |
| 46 | 34 | 127 | 45.72 | 1 |
| 47 | 43 | 127 | 19.47 | 1 |
| 48 | 33 | 127 | 25.54 | 2 |
| 20 krads | | | | |
| 1 | 29 | 127 | 36.55 | 1 |
| 2 | 34 | 127 | 23.81 | 2 |
| 3 | 19 | 127 | 6.36 | 2 |
| 4 | 14 | 127 | 6.84 | 2 |
| 5 | 36 | 127 | 55.18 | 3 |
| 6 | 17 | 127 | 12.04 | 1 |

*Mean of 30 observations.

TABLE IV. MEAN OF SOME EVALUATED AGRONOMIC CHARACTERISTICS IN $M_2$ GENERATION.

| | Panicles per plant (No.) | Yield per plant (g) | 100 grain weight (g) | Sterility (%) |
|---|---|---|---|---|
| Control | 27 a | 41.0 b | 2.3 a | 21.5 a |
| 15 krad | 24 a | 82.1 a | 2.5 a | 16.6 a |
| 20 krad | 24 a | 69.2 a | 2.3 a | 31.0 a |

* Values followed by the same letter are not statistically different, according to Duncan Test (5 %).

TABLE V. AGRONOMIC TRAITS FOR 52 SELECTED $M_3$ LINES.

| Line | Dose krad | Albino (%) | Height (cm) | Tillers No./m row | Empty grains (%) | Thrash (%) | Yield (t/ha) (12% moisture) | Blast response (0-9) |
|---|---|---|---|---|---|---|---|---|
| UN-9001 | 20 | 0 | 90 | 105 | 6.2 | 12 | 11.35 | 3 |
| UN-9002 | 15 | 0 | 85 | 120 | 6.3 | 14 | 12.00 | 3 |
| UN-9003 | 20 | 0 | 85 | 121 | 9.4 | 31 | 7.36 | 5 |
| UN-9004 | 20 | 0 | 80 | 118 | 6.8 | 19 | 9.22 | 3 |
| UN-9005 | 15 | 30 | 95 | 121 | 7.8 | 13 | 9.38 | 3 |
| UN-9006 | 20 | 20 | 80 | 105 | 8.1 | 26 | 8.51 | 3 |
| UN-9007 | 15 | 40 | 80 | 153 | 7.5 | 11 | 9.93 | 3 |
| UN-9008 | 15 | 0 | 75 | 118 | 6.4 | 25 | 9.27 | 3 |
| UN-9009 | 15 | 0 | 90 | 119 | 6.3 | 19 | 9.22 | 3 |
| UN-9010 | 15 | 10 | 85 | 112 | 5.9 | 8 | 7.42 | 3 |
| UN-9011 | 15 | 10 | 72 | 116 | 7.7 | 22 | 9.16 | 5 |
| UN-9012 | 15 | 0 | 90 | 136 | 14.0 | 12 | 7.47 | 3 |
| UN-9013 | 15 | 40 | 90 | 108 | 6.9 | 28 | 6.22 | 3 |
| UN-9014 | 15 | 0 | 90 | 139 | 9.5 | 16 | 9.00 | 3 |
| UN-9015 | 15 | 10 | 86 | 159 | 8.2 | 11 | 9.27 | 3 |
| UN-9016 | 15 | 0 | 8 | 131 | 3.4 | 21 | 57.15 | 3 |
| UN-9017 | 15 | 0 | 85 | 158 | 7.5 | 21 | 11.35 | 3 |
| UN-9018 | 15 | 0 | 85 | 116 | 6.8 | 17 | 6.49 | 3 |
| UN-9019 | 15 | 0 | 80 | 120 | 6.2 | 15 | 9.22 | 3 |
| UN-9020 | 20 | 0 | 85 | 115 | 6.5 | 9 | 7.36 | 5 |
| UN-9021 | 15 | 0 | 86 | 125 | 6.7 | 12 | 8.45 | 5 |
| UN-9022 | 15 | 0 | 85 | 131 | 6.4 | 11 | 8.62 | 3 |
| UN-9023 | 15 | 1 | 85 | 129 | 7.2 | 16 | 6.93 | 5 |
| UN-9024 | 15 | 2 | 85 | 150 | 5.2 | 5 | 10.96 | 3 |
| UN-9025 | 15 | 0 | 85 | 167 | 14.7 | 12 | 5.29 | 3 |
| UN-9026 | 15 | 1 | 90 | 134 | 7.5 | 27 | 9.27 | 7 |
| UN-9027 | 15 | 0 | 76 | 145 | 5.9 | 17 | 9.87 | 3 |
| UN-9028 | 15 | 0 | 90 | 125 | 7.0 | 16 | 8.35 | 5 |
| UN-9029 | 15 | 30 | 76 | 140 | 6.8 | 6 | 10.80 | 5 |
| UN-9030 | 15 | 1 | 85 | 130 | 7.4 | 25 | 7.58 | 3 |
| UN-9031 | 15 | 40 | 88 | 125 | 4.6 | 8 | 8.18 | 5 |
| UN-9032 | 15 | 30 | 90 | 115 | 3.7 | 31 | 6.87 | 3 |
| UN-9033 | 15 | 1 | 75 | 115 | 4.3 | 9 | 7.36 | 3 |
| UN-9034 | 15 | 0 | 76 | 130 | 6.3 | 11 | 8.89 | 7 |
| UN-9035 | 15 | 0 | 80 | 140 | 6.1 | 13 | 9.98 | 3 |
| UN-9036 | 15 | 0 | 75 | 115 | 5.4 | 8 | 7.53 | 3 |
| UN-9037 | 15 | 0 | 78 | 133 | 5.6 | 0 | 9.11 | 3 |
| UN-9038 | 15 | 0 | 90 | 187 | 4.9 | 13 | 13.15 | 5 |
| UN-9039 | 15 | 30 | 90 | 131 | 4.8 | 18 | 9.00 | 3 |
| UN-9040 | 15 | 0 | 90 | 140 | 7.7 | 31 | 9.00 | 4 |
| UN-9041 | 15 | 0 | 75 | 124 | 6.1 | 7 | 9.27 | 5 |
| UN-9042 | 15 | 40 | 85 | 153 | 11.1 | 13 | 10.15 | 3 |
| UN-9043 | 15 | 5 | 90 | 163 | 4.7 | 33 | 10.42 | 5 |
| UN-9044 | 15 | 5 | 90 | 143 | 6.5 | 23 | 9.87 | 3 |
| UN-9045 | 15 | 0 | 85 | 123 | 5.9 | 29 | 9.22 | 2 |
| UN-9046 | 15 | 0 | 83 | 159 | 6.7 | 33 | 10.75 | 3 |
| UN-9047 | 15 | 30 | 80 | 118 | 7.0 | 6 | 8.67 | 5 |
| UN-9048 | 15 | 1 | -- | 131 | 8.4 | 7 | 8.73 | 3 |
| UN-9049 | 20 | 0 | 70 | 151 | 6.4 | 17 | 9.33 | 3 |
| UN-9050 | 15 | 0 | 90 | 128 | 4.3 | 9 | 8.13 | 3 |
| UN-9051 | 15 | 0 | 80 | 150 | 7.5 | 9 | 11.45 | 3 |
| UN-9052 | 15 | 40 | 80 | 121 | 7.5 | 16 | 8.73 | 3 |
| CR-1113 | 0 | 0 | 90 | 127 | 21.5 | 12 | 4.03 | 8 |

TABLE VI. RANGES AND MEAN VALUES OF SOME AGRONOMIC FEATURES OF THE 52 $M_3$ MUTANT LINES.

| Character | Control CR-1113 (mean) | Range | Mean |
|---|---|---|---|
| Number of productive tillers | 125 | 105-187 | 131.0 |
| Percent of empty grains | 5 | 3.4-4.7 | 6.8 |
| Number of grains per panicle | 190 | 118.5-242.1 | 152.2 |
| Panicle length (cm) | 25 | 18.6-26.4 | 23.8 |
| Plant height (cm) | 80 | 72-90 | 83.8 |
| Flag leaf length (cm) | 26 | 19.5-32.6 | 25.3 |
| Low leaf length (cm) | 34 | 23.5-43.0 | 35.2 |
| Percent of thrash | 8 | 0-33 | 15.8 |

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# INDUCED MUTAGENESIS IN RICE IN COLOMBIA

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**Abstract**

Three rice varieties (CICA-8, Oryzica-I and Oryzica-2) were treated with gamma rays to induce blast resistant mutants. Mutant lines were selected from CICA-8 and *Oryzica*-1 that in the preliminary test showed yield differences between the original variety and the selected lines. Although blast infection scores were similar for the checks and the mutant lines, the lines are useful for non-endemic blast areas because of their good agronomic traits.

## 1. INTRODUCTION

Blast, caused by *Pyricularia grisea,* is the most important disease that limits rice production in many countries. In Colombia, there are areas where blast spreads rapidly under favorable conditions and is highly destructive. Therefore, it is important to develop blast resistant varieties. However, the blast fungus is highly variable and the resistance in newly released varieties breaks down after two or three years of cultivation. As a result, new blast resistant varieties must be continuously developed.

The genetic base of the varieties released in Colombia during the period 1971-1989 is very narrow [1]. The sources for blast resistance are limited. Although several varieties, such as Tetep, Tadukan, Dissi Hatif and Colombia-1 have been resistant in repeated tests at IRRI (International Rice Research Institute) and in many locations around the world through the IRBN (International Rice Blast Nursery), only Colombia-1 has shown resistance in our conditions, which implies that most of the segregating populations have Colombia-1 as the blast resistant source.

The Rice Programme has used mutation techniques in an effort to obtain semidwarf mutants of the tall donor parents to make them more suitable for crosses. In the current project, the major objective is to identify and use stable resistance to the major pests through different approaches like mutation breeding to develop blast resistant mutants which are agronomically useful.

## 2. MATERIALS AND METHODS

*Genotypes used*

CICA-8. This variety was released by the Rice Programme of Colombia in 1978. It has a high yield potential and good adaptability, particularly under upland conditions, but after 3 years of cultivation it became susceptible to blast. Also, it is susceptible to lodging under irrigated conditions when the water table is high. CICA-8 has been nominated as a variety in Bolivia, Brazil, Guatemala, Venezuela and other countries. The pedigree is P918-25-14-2-3-1B from the cross CICA-4//IR665-23-3/Tetep.

Oryzica-1. This variety was released by the Rice Programme of Colombia in 1982 because of good performance under both upland and lowland conditions. It is popular because of good grain quality. In 1991, Oryzica-1 was cultivated on 80% of the rice area in Colombia. It is now susceptible to blast and lodging. The pedigree of Oryzica-1 is P1429-8-9m-2-1B. It originated from a multiple cross. It has been nominated as a variety in other South-American countries.

Oryzica-2. This variety was released by the Rice Programme of Colombia in 1984 because of high yield potential and tolerance to blast. It has poor grain quality because of white-belly chalk and low milling recovery. The pedigree is P2023 F4-74-2-1B from the cross BG90-2//CICA-8/CICA-7.

*Treatments*

Seeds of the three varieties were treated (30 g per variety and treatment) with gamma rays 20, 25 and 30 krads (200, 250 and 300 Gy) and with sodium azide $1x10^{-3}$ and $3x10^{-3}$ M for 8 hours.

The radiation treatments were done at the Nuclear Institute of Colombia with a $^{60}Co$ radiation source. The treated seeds were sown at La Libertad, Villavicencio. Three panicles were individually harvested from each of 4218 $M_1$ plants. A sample of seed from each $M_1$ plant was sown in a row 5 m long.

## 3. RESULTS AND DISCUSSION

In all irradiated populations of the $M_1$ generation as well as in the accompanying non-irradiated control populations, a high level of sterility was observed, apparently as a result of low temperatures during flowering. The sterility data were recorded at maturity (Table I). The sodium azide treatments caused a high level of sterility (Table I). In the $M_2$ generation populations derived from sodium azide treatments, dwarf and sterile plants were observed but they were not considered of any value for the breeding programme.

TABLE I. EFFECT OF GAMMA RAYS AND SODIUM AZIDE TREATMENTS ON $M_1$ STERILITY.

| Treatment | Sterility | | |
|---|---|---|---|
| | Oryzica-1 | Oryzica-2 | CICA-8 |
| Gamma rays | | | |
| 0 (Check) | 23 | 26 | 13 |
| 20 krad | 37 | 53 | 40 |
| 25 krad | 57 | 41 | 66 |
| 30 krad | 60 | 55 | 58 |
| Sodium azide | | | |
| 0.001 M | - | 91 | 90 |
| 0.003 M | - | 90 | 80 |

The $M_2$ populations derived from irradiation treatments consisted of 4218 five meter rows of the three varieties grown at La Libertad under irrigated conditions. Blast infection on the $M_2$ plants of CICA-8 and Oryzica-1 forty days after germination was severe, and disease-free plants were identified. During flowering, the plants were evaluated for neck blast and the resistant plants were harvested for evaluation in the next generation. Also, earlier plants of CICA-8 were harvested separately for later evaluation. In the $M_2$ populations of Oryzica-2, selections were based on phenotypic appearance and grain translucency and plants were harvested individually.

From the three varieties in $M_2$, 527 individual plants were harvested and subsequently sown in 3-row x 5 m plots to constitute $M_3$ generation lines, which were evaluated for blast tolerance and agronomic characters. The lines that had lower blast infections than the controls and the earlier maturing selections from CICA-8 were harvested. A total of 145 lines were selected, including 103 from Oryzica 2, 21 from CICA-8, and 19 from Oryzica-1.

One hundred grams of the 103 lines from Oryzica-2 were milled and evaluated for chalkiness. All were discarded because none showed any less chalk than the control, Oryzica-2.

In the $M_4$ generation, the 21 lines of CICA-8 and the 19 lines of Oryzica-1 plus the controls were sown and evaluated at La Libertad for blast tolerance, earliness, lodging and grain quality. From the 40 lines, four lines of CICA-8 and three lines of Oryzica-1 were evaluated for yield and other traits in a preliminary trial (Table II).

TABLE II. PERFORMANCE AND SOME CHARACTERISTICS OF THE MUTANTS FROM CICA-8 AND ORYZICA-1, VILLAVICENCIO, COLOMBIA, 1990.

| Mutant lines | Blast* (leaf) | Earliness | White belly* | Amylose % | GT** | Yield (kg/ha) |
|---|---|---|---|---|---|---|
| Oryzica-1 (Check) | 3 | 0 | 0.6 | 28 | L | 2807 |
| Mut-Oryzica 1-1 | 2 | 0 | 0.8 | 27 | L | 3715 |
| Mut-Oryzica 1-2 | 2 | -5 | 0.4 | 28 | L | 3348 |
| Mut-Oryzica 1-3 | 4 | -5 | 0.4 | 27 | L | 4090 |
| CICA-8 (Check) | 4 | 0 | 2.4 | 26 | H/I | 5589 |
| Mut-CICA 8-1 | 5 | -10 | 2.8 | 26 | H/I | 4248 |
| Mut-CICA 8-2 | 5 | -8 | 4.4 | 26 | H/I | 3798 |
| Mut-CICA 8-3 | 5 | -15 | 3.2 | 27 | H/I | 4798 |
| Mut-CICA 8-4 | 4 | 0 | 2.8 | 27 | I/H | 4881 |

* Standard evaluation system for rice developed by IRRI and CIAT.
** GT=Gelatinization temperature, L = Low, I = Intermediate, H = High.

The mutant lines derived from Oryzica-1 produced higher yields than the control at Villavicencio in 1990 (Table II), but any differences in blast reactions and grain quality characteristics were slight. Two of the mutants were five days earlier than Oryzica-1.

The mutants derived from CICA-8 were all lower yielding than the original variety, and none showed improvement in the reaction to leaf blast. Some differences in grain translucency were observed between the mutants and the control. Although the CICA-8 mutants were lower yielding than the control, three of them were eight to fifteen days earlier, which may be important to the farmer in facilitating crop rotations, scheduling sowing and harvesting operations, and reducing the probability of encountering undesirable weather conditions for the crop.

Three Oryzica-1 mutants and two CICA-8 mutants were included in a replicated yield trial at La Libertad in 1991 (Table III). One Oryzica-1 mutant, Line No. 30575, produced a statistically significant higher yield than the original parent. No other yield differences in the test were statistically significant.

TABLE III. PERFORMANCE OF FIVE $M_5$ RICE MUTANTS IN LA LIBERTAD, 1991.

| Mutant lines | Line No. | Yield, kg/ha* |
|---|---|---|
| Mut-Oryzica 1-2 | 30575 | 4250 a |
| Mut-Oryzica 1-3 | 30576 | 3792 ab |
| Mut-CICA 8-1 | 30577 | 3550 ab |
| Mut-CICA 8-3 | 30578 | 3917 ab |
| Mut-CICA 8-4 | 30579 | 4208 ab |
| Oryzica-1 (Check) | | 3500 b |
| CICA-8 (Check) | | 3958 ab |

* Level of significance = 5%.

## 5. CONCLUSIONS

- The induced mutations approach is useful for inducing genetic variability in some traits.
- The selected mutants should be evaluated in different areas to determine their yield potential and stability.
- It is important to utilize effective screening techniques for blast reaction to assure that disease-free plants are truly resistant, rather than susceptible plants that escaped infection.
- Low doses of radiation should be used to induce small changes and recover useful mutants.
- Use well adapted varieties and look for dwarfness, earliness and disease resistance.

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# RICE IMPROVEMENT THROUGH MUTATION INDUCTION WITH GAMMA RAYS AND SODIUM AZIDE

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**Abstract**

The objective of this research in rice (*Oryza sativa* L.) was to induce mutations for semidwarfism, early maturity, high yield potential and tolerance to *Pyricularia grisea* in the rice variety Pico Negro. Seeds were treated with sodium azide and gamma rays alone and in combination at various doses. In the $M_1$ generation the number of chlorophyll deviations was recorded for the various treatments in order to select the optimum one, which proved to be 2.5 x $10^{-3}$ M $NaN_3$ + 2.5 krad (25 Gy). In the $M_2$ generation, mutations were obtained for morphological and chlorophyll characters, plant height, and earliness. The stability of the mutants selected in the $M_2$ generation was confirmed in the $M_3$ generation. A total of 148 mutants were selected.

## 1. INTRODUCTION

Rice is one of the basic cereals in the diet of the Guatemalan population. There are native varieties of rice with good yields and tolerance to *Pyricularia grisea*, but they are susceptible to lodging. Because of the rapid growth of the population in Guatemala, it is important to increase rice production as quickly as possible in order to satisfy the expanding needs.

The objective of this research project is to utilize a combination of mutagens (gamma rays and sodium azide) to induce semidwarf, early maturing, blast resistant, high yielding mutants that can be distributed to growers as new varieties or that can be used in the rice breeding programmes in the country.

## 2. MATERIALS AND METHODS

*Location of the field experiment:*

The research was developed by the Agricultural Section of the General Directorate for Nuclear Energy with the cooperation of the Rice Breeding Programme of the National Institute of Science and Agricultural Technology. The experiment was established at the Cristina Production Center in Izabal, located in the Tropical Humid ecological zone on the Atlantic coast of Guatemala.

*Material*

The native variety Pico Negro was used in this study. It was cultivated by farmers in the north region of the country years ago. It is adapted to most of the constraints of the region and has good grain quality, but it is very tall and susceptible to lodging.

*Treatments*

The treatments used were the following:

1. Control
2. 5 krad
3. 10 krad
4. 15 krad
5. 20 krad
6. 25 krad
7. 30 krad
8. 35 krad
9. $1.0 \times 10^{-3}$ M $NaN_3$
10. $2.5 \times 10^{-3}$ M $NaN_3$
11. $5.0 \times 10^{-3}$ M $NaN_3$
12. 25 krad + $1.0 \times 10^{-3}$ M $NaN_3$
13. 25 krad + $2.5 \times 10^{-3}$ M $NaN_3$
14. 25 krad + $5.0 \times 10^{-3}$ M $NaN_3$
15. $1.0 \times 10^{-3}$ M $NaN_3$ + 2.5 krad
16. $2.5 \times 10^{-3}$ M $NaN_3$ + 2.5 krad
17. $5.0 \times 10^{-3}$ M $NaN_3$ + 2.5 krad

*Methods*

Rice seeds of the variety Pico Negro were treated with sodium azide in concentrations of $1.0 \times 10^{-3}$ M, $2.5 \times 10^{-3}$ M and $5.0 \times 10^{-3}$ M in combination with pre- and post-treatments with gamma rays of $^{60}Co$ in doses of 2.5 krad and 25 krad, respectively. The following method was used to select the treatments: the chemical mutagen was applied through soaking the seeds at ambient temperature and shaking continuously for 18 hours. The seeds were then washed in water to eliminate the mutagen and dried on paper towel to eliminate surface moisture [1-3]. The seeds were sown to measure the percent reduction in germination and height, and based on the results, two treatments were selected.

*The $M_1$ generation*

Six thousands seeds of each treatment were sown in 60 rows, with 100 seeds/row. There was one control for every 10 rows in the experimental area. During the $M_1$ generation, somatic effects were observed. Later, the surviving normal plants were harvested individually, taking two panicles from the main culm. One panicle from each plant collected in the $M_1$ generation was sown individually in a pot to determine the percentage of chlorophyll mutants in the two treatments, in order to select the one that caused the most mutants for continuing into the $M_2$ generation [1,4].

*The $M_2$ generation*

The selection of the treatments for establishing the $M_2$ generation was based on the analysis of the $M_1$ data. The $M_2$ populations consisted of panicle rows 2 m in length, each row sown with 20 grains from each harvested $M_1$ panicle. Rows of the controls were planted at intervals throughout the experimental area. The total $M_2$ population was 48 000 plants. During the crop season the plants were observed, to identify semidwarf and earlier types. The yield was measured on an individual plant basis, collecting all mutant seeds from the progeny. In addition, one normal plant was collected from each progeny row to serve as a non-treated control in the next generation [1,4,5].

*The $M_3$ generation*

In this generation, seeds harvested from short plants identified in the $M_2$ generation were sown, 5 grains per plant in 3 meter rows. During this test, the plant population was carefully observed to verify the genetic stability of the mutants selected in the $M_2$ [1,4,5].

## 3. RESULTS AND DISCUSSION

In the greenhouse, the 20-25 krad dose range decreased germination 40% and seedling height 60%. In the field, the reduction in surviving plants was 70% and seedling height 70%. In the single and combined treatments (gamma rays and sodium azide), the treatments 1.0 x $10^{-3}$ M $NaN_3$ + 2.5 krad and 2.5 x $10^{-3}$ M $NaN_3$ + 2.5 krad reduced the height of the seedlings 29-37% and the surviving plants 28-51% (Table I and Figure 1). The treatment 2.5 x $10^{-3}$ M $NaN_3$ + 2.5 krad produced more chlorophyll mutants than 1.0 x $10^{-3}$ M $NaN_3$ + 2.5 krad (Table II). The same treatment also resulted in mutants that were shorter and earlier than the parent variety.

TABLE I. EFFECTS OF SINGLE AND COMBINED TREATMENTS OF SODIUM AZIDE AND GAMMA RAYS IN THE $M_1$ GENERATION OF PICO NEGRO RICE.

| Treatment | Seedling survival (%) | Stand reduction (%) | Seedling growth (%) | Growth reduction (%) |
|---|---|---|---|---|
| Control | 100 | 0 | 100 | 0 |
| 1.0 x $10^{-3}$ $NaN_3$ | 89 | 11 | 94 | 6 |
| 2.5 x $10^{-3}$ $NaN_3$ | 82 | 18 | 84 | 16 |
| 5.0 x $10^{-3}$ $NaN_3$ | 60 | 40 | 92 | 8 |
| 25 krad + 1.0 x $10^{-3}$ M $NaN_3$ | 30 | 70 | 90 | 10 |
| 25 krad + 2.5 x $10^{-3}$ $NaN_3$ | 16 | 84 | 47 | 53 |
| 25 krad + 5.0 x $10^{-3}$ $NaN_3$ | 15 | 85 | 30 | 70 |
| 1.0 x $10^{-3}$ M $NaN_3$ + 2.5 krad | 72 | 28 | 71 | 29 |
| 2.5 x $10^{-3}$ M $NaN_3$ + 2.5 krad | 49 | 51 | 63 | 37 |
| 5.0 x $10^{-3}$ M $NaN_3$ + 2.5 krad | 32 | 68 | 74 | 26 |

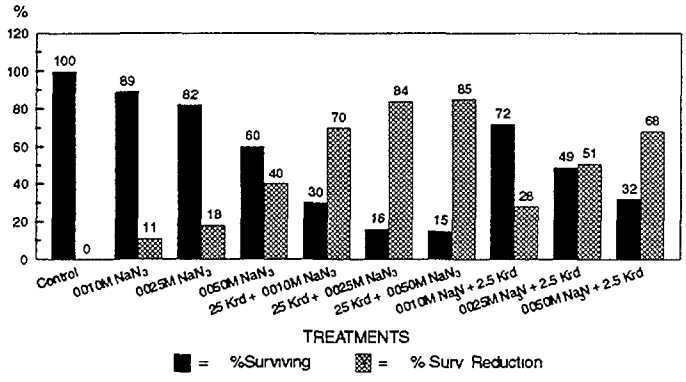


*FIG. 1. Physiological effects in $M_1$ of simple and compound mutagenic treatments (gamma rays and sodium azide) in Pico Negro.*

In the $M_3$ generation, the genetic stability of the $M_2$ mutants was confirmed. The mutants included shorter plants, different grain color, earlier maturing types (one as much as 20 days earlier), and chlorophyll types (Table III).

TABLE II. CHLOROPHYLL MUTANTS PER 100 $M_2$ PLANTS.

| Treatment | Chlorophyll mutants, % |
|---|---|
| Control | 0 |
| 2.5 x $10^{-3}$ $NaN_3$ + 2.5 krad | 23 |
| 1.0 x $10^{-3}$ $NaN_3$ + 2.5 krad | 7 |

TABLE III. FREQUENCY OF MUTANT TYPES IN THE $M_3$ GENERATION OF PICO NEGRO.

| Character | Number of mutants | Proportion of all mutant lines (%) |
|---|---|---|
| Dwarf | 52 | 35.12 |
| Semidwarf | 24 | 16.21 |
| Intermediate height | 57 | 38.51 |
| Chlorophyll deficiencies | 5 | 3.38 |
| Earliness | 3 | 2.03 |
| Morphological variations | 3 | 2.03 |
| Dwarf | 2 | 1.35 |
| Brown hull color | 2 | 1.35 |
| TOTAL | 148 | 100.00 |

## 3. CONCLUSIONS

The combined treatment 2.5 x $10^{-3}$ M $NaN_3$ + 2.5 krad caused the greatest percentage of chlorophyll mutants in the greenhouse. In the field it induced mutants for earliness (one even 20 days earlier), a different hull color, and short plants classified as dwarf, semidwarf and intermediate. In the evaluation of the $M_2$ and $M_3$ generations, it was concluded that selection in the $M_2$ generation was effective and the mutants were confirmed in the $M_3$ generation.

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# INDUCTION OF SHORT CULM MUTANTS IN RICE BY SODIUM AZIDE

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## 1. INTRODUCTION

Sodium azide ($NaN_3$) is well known for its high mutagenic effect in several crops, depending on treatment conditions [1,6]. The technical and practical evaluation of a chemical mutagen is generally based on its solubility in water, toxicity, and reaction to and with biological material. In these respects, sodium azide is an excellent chemical mutagen, with its high solubility in water, strong reaction with and low toxicity to biological materials as compared with alkylating compounds that are usually used for mutation induction in plants. In addition to these advantages, sodium azide gives in various crops higher chlorophyll mutation frequencies than ionizing radiations and alkylating compounds such as ethyl methanesulphonate (EMS), ethylene imine (EI), and diethyl sulphate (DES), which are frequently used [2,3-6].

## 2. MATERIALS AND METHODS

Dormant and fertile seeds of the upland rice cultivar IAC 1246 were used. The sodium azide treatments are presented in Table I. All sodium azide treatments were conducted at 25°C in a controlled environment chamber. The treated seeds were sown separately in wooden boxes and all seedlings were transplanted individually into the field, with plants spaced 10 cm apart in rows spaced 40 cm apart. Three panicles were harvested from the primary tillers of each surviving $M_1$ plant, and 3 to 5 fertile $M_2$ seeds were collected at random from each harvested panicle. Within each treatment, all $M_2$ seeds were bulked and sown in seedbeds. After one month, only morphologically normal $M_2$ seedlings were transplanted individually into the field at the same spacing as with the $M_1$ generation.

The height of the tallest culm of each $M_2$ plant was measured before harvest, and the selection of short culm mutants was conducted with an average selection pressure of about 4%. The $M_3$ seeds were collected individually from each selected $M_2$ plant and sown in progeny rows in the field. Plant height was measured in all surviving fertile plants. Selection for plants with short stature and normal appearance was conducted within each progeny row.

The selected $M_3$ plants were harvested separately and sown in progeny rows in the field in a randomized block design with 7 replications. Each replication consisted of 10 plants with a 10 cm spacing between plants and 40 cm between rows. In addition to plant height, which consisted of the average height of 70 $M_4$ plants per progeny, all fertile grains of each line were harvested and weighed. Based on short plant height and grain yield per plant, 10 lines were selected in the $M_4$ generation.

## 3. RESULTS AND DISCUSSION

Table II includes data on the number of $M_1$ seeds treated, the number of $M_2$ plants that were transplanted and survived to maturity, and the number of $M_2$ plants, $M_3$ lines, and $M_4$ lines that were selected, based on shorter stature and higher grain yield per plant.

TABLE I. SODIUM AZIDE ($NAN_3$) TREATMENT CONDITIONS FOR UPLAND RICE VARIETY IAC 1246.

| Treatment | Pre-soaking duration (hr) | Concentration (x $10^{-3}$M) | Buffer solution pH | Treatment duration (hr) |
|---|---|---|---|---|
| 1 | 5 | 5 | 3.0 | 8 |
| 2 | 5 | 10 | 3.0 | 8 |
| 3 | 5 | 5 | 4.0 | 8 |
| 4 | 5 | 10 | 4.0 | 8 |
| 5 | 8 | 5 | 3.0 | 8 |
| 6 | 8 | 10 | 3.0 | 8 |
| 7 | 8 | 5 | 4.0 | 8 |
| 8 | 8 | 10 | 4.0 | 8 |
| 9 | 5 | 1 | 3.0 | 8 |
| 10 | 5 | 1 | 4.0 | 8 |
| Control | - | - | - | - |

TABLE II. NUMBER OF TREATED $M_1$ SEEDS AND SELECTED $M_2$, $M_3$, AND $M_4$ PLANTS FOR SHORT CULM LENGTH.

| Treatment | No. $M_1$ seeds treated | $M_2$ plants, No. | | | Selected plants, No. | |
|---|---|---|---|---|---|---|
| | | Transplanted | Survived | Selected | $M_3$ | $M_4$ |
| 1 | 1200 | 945 | 314 | 16 | 9 | 4 |
| 2 | 400 | 123 | 81 | 7 | 2 | 0 |
| 3 | 1200 | 1202 | 340 | 16 | 5 | 0 |
| 4 | 400 | 173 | 108 | 6 | 2 | 0 |
| 5 | 400 | 435 | 123 | 6 | 1 | 0 |
| 6 | 400 | 29 | 23 | 15 | 7 | 4 |
| 7 | 400 | 382 | 114 | 3 | 3 | 1 |
| 8 | 400 | 384 | 116 | 6 | 2 | 0 |
| 9 | 800 | 1500 | 493 | 14 | 0 | 0 |
| 10 | 800 | 1307 | 141 | 4 | 0 | 0 |
| Control | 400 | 1757 | 421 | 3 | 1 | 1 |
| TOTAL | 6800 | 8237 | 2274 | 96 | 33 | 10 |

In the $M_2$ generation 96 plants were selected, about 4%. All of the selected plants were at least 7 cm shorter than the control. In the $M_3$ generation, selection was for shorter plants, normal plant type and good fertility. Thirty-three $M_3$ plants were selected and sown directly in the field in order to verify their shorter height and to evaluate their grain productivity per plant. Ten $M_3$ progenies were selected in the $M_4$ generation. All of the mutant lines were shorter than the control, varying from very slightly to considerably shorter. Nine of the mutants were higher yielding than the control, with the yield increases ranging from 5 to 70%. One mutant yielded the same as the control. Evaluation of the selected mutant lines is continuing with the objective of identifying promising mutant lines that are short and yield as well or better than IAC 1246, the original cultivar.

TABLE III. RELATIVE CULM LENGTH AND GRAIN YIELD PER PLANT OF THE SELECTED $M_4$ PROGENIES EVALUATED IN THE $M_4$ GENERATION.

| Selected $M_4$ progenies | Culm length (%) | Grain yield/plant (%) |
|---|---|---|
| Control | 100.0 | 100.0 |
| 1 | 76.4 | 129.5 |
| 2 | 96.1 | 105.1 |
| 3 | 97.4 | 144.9 |
| 4 | 82.3 | 170.5 |
| 5 | 82.6 | 129.5 |
| 6 | 97.7 | 152.6 |
| 7 | 70.6 | 137.2 |
| 8 | 78.8 | 170.5 |
| 9 | 99.0 | 110.3 |
| 10 | 75.8 | 100.0 |

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# CALLUS INDUCTION AND PLANT REGENERATION FROM ANTHER CULTURE OF RICE

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**Abstract**

Anthers of five mutant lines of rice derived from the variety ICTA VIRGINIA and five derived from the variety PRECOZICTA as well as the two parental varieties (both varieties *indica*) containing uninucleate pollen grains, were cultured on a $N_6$ agar medium with 5% sucrose concentration and supplemented with 3 mg/l NAA, 1 mg/l kinetin and 1 mg/l 2,4-D.

The proportion of anthers that produced calli varied from 0% for the mutant lines 881 and 940 to 1.9% for the parental variety Precozicta. The calli were transferred to $N_6$ medium with 3% sucrose concentration and supplemented with 0.5 mg/l NAA and 1 mg/l kinetin and plantlets developed in 2-5 weeks. A total of 153 green plantlets were obtained.

## 1. INTRODUCTION

In 1987, a mutation breeding program in rice was started in Guatemala, with two varieties which became susceptible to the blast disease, caused by *Pyricularia grisea*. Ten of the best mutant lines obtained and their parental varieties were evaluated for their callus forming capacity and plant regeneration. The difficulty in anther culture of *indica* rice (*Oryza sativa* L.) has been the low capacity of the pollen grains to produce callus and its subsequent low differentiation rate into plants [1-5].

Recent basic studies have focused on the evaluation of *indica* varieties for their response to anther culture, the genetics of its response, and methods to increase its efficiency. Results indicated that callus induction and plant regeneration abilities are controlled by recessive genes, with no involvement of the cytoplasm. Also that anther culture responsiveness of a recalcitrant *indica* variety can be improved through hybridization with a responsive cultivar [6].

Androgenesis is closely related to the genome of the species and differs among species and varieties [7]. Significant differences among genotypes tested under the same conditions have been noted [8].

## 2. MATERIALS AND METHODS

The varieties and lines of rice used for this study are presented in Table I. All plants were grown in the greenhouse of the Nuclear Energy General Directorate during February-May 1992. The tillers of the plants were collected when the inflorescences were still enclosed within the sheath. Florets were surface sterilized in 2% sodium hypochlorite for 20 minutes, and then rinsed thoroughly in sterile distilled water. Anthers containing uninucleate pollen grains were removed from the florets

and transferred to the surface of agar solidified $N_6$ medium with 5% of sucrose and supplemented with 3 mg/l NAA, 1 mg/l kinetin and 1 mg/l 2,4-D. The cultures were incubated in a growth chamber at a constant temperature of 27°C, in the dark.

TABLE I. FREQUENCY OF CALLUS FORMATION IN ANTHER CULTURES OF 12 GENOTYPES OF RICE.

| Material | Anthers plated (No.) | Anthers forming calli (%) |
|---|---|---|
| Parent PRECOZICTA | 1225 | 1.88 |
| Mutant PI1146 | 1700 | 0.82 |
| Mutant PI2032 | 425 | 1.65 |
| Mutant PI2097 | 1125 | 0.89 |
| Mutant PI1736 | 1000 | 0.80 |
| Mutant PI1796 | 1000 | 1.10 |
| Parent ICTA VIRGINIA | 875 | 0.46 |
| Mutant V620 | 3100 | 0.64 |
| Mutant V1001 | 700 | 0.71 |
| Mutant V881 | 1750 | 0.00 |
| Mutant V568 | 625 | 0.96 |
| Mutant V940 | 625 | 0.00 |

Two to three weeks after calli initiation, they were transferred to $N_6$ medium with 3% sucrose, 0.5 mg/l NAA and 1 mg/l kinetin, to induce organ differentiation. The cultures were maintained in the same condition as for callus induction except that they had a 16 hours photoperiod. After the root system had developed, the plants were transplanted into pots in greenhouse.

## 3. RESULTS AND DISCUSSION

There were variations in the twelve genotypes studied with regard to the capacity of their pollen to produce calli (Table I), although the frequencies of calli production were very low. In the parental variety PRECOZICTA, the frequency of anthers that produced calli was 1.88%, in the mutant PI2032 derived from PRECOZICTA it was 1.65% and 1.1% for the mutant PI1796 also derived from PRECOZICTA. All other frequencies were below 1%, and 0% for mutants V881 and V940, both derived from the parental variety ICTA VIRGINIA. After the calli were transferred to the differentiation medium, plants developed in 2-5 weeks. A total of 153 green plants was obtained (Table II).

Anther culture of *indica* rice is very difficult. The induction of green plants is usually less than 1% (induction frequency = the number of green plants/the number of inoculated anthers x 100) as described by Zhu Deyao and Pan Xigan [9]. They obtained 12.8% average induction frequency of callus and 1.8% average induction frequency of green plants of hybrid rice spikes from Shan-You 2 (Zhen Shan 97A/$IR_{24}$) $F_1$ in similar conditions. For the present research, the mean induction frequency of callus was 0.8%, the mean percent of calli forming green plants was 0.6 and the mean induction frequency 1.2 green plants/100 plated anthers.

TABLE II. FREQUENCY OF ORGAN FORMATION AND EFFICIENCY IN ANTHER DERIVED PLANTS OF RICE.

| Material | Calli (No.) | Calli forming plants (%) | Green plants (No.) | Efficiency (%) |
|---|---|---|---|---|
| PRECOZICTA | 23 | 30.4 | 126 | 10.3 |
| Mutant P2032 | 7 | 14.3 | 11 | 0.6 |
| ICTA VIRGINIA | 4 | 25.0 | 5 | 0.4 |
| Mutant V620 | 20 | 5.0 | 11 | 2.6 |

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# RADIATION EFFECT IN ANTHER CULTURES OF RICE VARIETY KRISPO-38

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**Abstract**

Seeds of rice variety Krispo-38 were irradiated with 0, 100, 200, 300 and 400 Gy and sown in the greenhouse. Anthers from plants of each treatment containing uninucleate pollen grains were collected and cultured on an $N_6$ agar medium with 5% sucrose concentration and supplemented with 3 mg/l NAA, 1 mg/l kinetin and 1 mg/l 2,4-D. The pollen grains were induced to develop calli. The proportion of anthers that produced calli varied from 0.8% for the 400 Gy treatment to 3.3% for the control. The calli were transferred to $N_6$ medium with 3% sucrose concentration and supplemented with 0.5 mg/l NAA and 1 mg/l kinetin. The proportion of calli that produced green plants varied from 1.9% for the 300 Gy treatment to 10.5% for the 200 Gy one. Plants developed in 2-5 weeks, 101 plants were obtained.

## 1. INTRODUCTION

Extensive efforts have been made to improve anther culture efficiency in *indica* rice. It has been proven that improvement in the anther culture ability of recalcitrant varieties or species can be obtained using radiation [1]. Seed irradiation with 100 to 300 Gy using gamma rays induced plant regeneration following anther culture of recalcitrant *indica* varieties such as IR8, IR42, IR50 and IR64 [2].

Breaking through radiation the linkage between the non-culturability character and the agronomic characters inherent to the *indica* rice, it may be possible to express the character for culturability. Zapata and Aldemita [3] induced androgenesis of an *indica* rice variety, "Basmati-370", by irradiating seeds with 200 and 250 Gy gamma rays. Increasing the culturability of recalcitrant but high yielding varieties is important for possible production of gametoclonal variants which would be resistant to diseases and pests and tolerant to extreme environmental conditions.

## 2. MATERIALS AND METHODS

Rice variety Krispo-38 was used for this study. Irradiation from a source of $^{60}Co$ was applied to the seeds in five different doses: 0, 100, 200, 300 and 400 Gy. All plants were grown under greenhouse conditions in the Nuclear Energy General Directorate, during February-May 1992. The first few tillers of the plants were collected when the inflorescences were still enclosed within the sheath. Florets were surface sterilized in 2% sodiumhypochlorite for twenty minutes, and then rinsed thoroughly in sterile distilled water. Anthers containing uninucleate pollen grains were removed from the florets and transferred to the surface of $N_6$ agar solidified medium with 5% sucrose concentration and supplemented with 3 mg/l NAA, 1 mg/l kinetin and 1 mg/l 2,4-D. The cultures were incubated in a growth chamber without light at a constant temperature of 27°C.

Two to four weeks after calli initiation, the calli were transferred to $N_6$ agar solidified medium with 3% sucrose concentration and supplemented with 0.5 mg/l NAA and 1 mg/l kinetin and incubated with a 16 hours photoperiod at 30°C. After the root system developed, the plants were transplanted into pots in greenhouse.

## 3. RESULTS AND DISCUSSION

Radiation in the applied doses was not effective in inducing callus formation. The untreated check was the most responsive in producing calli, 3.3%. All irradiated treatments were below 1.8%. However, radiation increased the capacity of the calli to regenerate green plants. Thus, 10.5% and 7.7% green plant regeneration were obtained for the 200 Gy and 100 Gy treatments respectively, while all other treatments were below 2.5% (Table I).

TABLE I. ANTHER CULTURE RESPONSE OF IRRADIATED RICE KRISPO-38.

| Treatment (Gy) | Anthers plated No. | Calli formed | | Calli | | | Green plants produced |
|---|---|---|---|---|---|---|---|
| | | | | Transferred | With green plants | | |
| | | No. | (%) | No. | No. | (%) | |
| 0 | 4425 | 146 | 3.3 | 146 | 3 | 2.0 | 10 |
| 100 | 3925 | 52 | 1.3 | 52 | 4 | 7.7 | 38 |
| 200 | 4075 | 38 | 0.9 | 38 | 4 | 10.5 | 20 |
| 300 | 6025 | 104 | 1.7 | 104 | 2 | 1.9 | 25 |
| 400 | 5325 | 42 | 0.8 | 42 | 1 | 2.4 | 8 |

The efficiency of green plant production was highest in the 100 Gy treatment, 0.97 green plants/100 plated anthers. The second highest was the 200 Gy treatment, with 0.49%. Lowest was 400 Gy with 0.15%. The control (0 Gy) was not efficient in green plant production, with 0.22%.

These results are low if we compare them with the best results reported with this method in rice: 31.2 green plants/100 plated anthers [4], 61.8 green plants/100 calli and 28.3 calli/100 plated anthers [5], 414.3 green plants/100 calli and 678.5 calli/100 plated anthers [6], but according to Ochoa [7], androgenesis is closely related to the genetics of species and its capacity may differ even between species or varieties. Similar results were found by Zapata and Aldemita [3], where irradiation decreased callus production efficiency but increased green plant regeneration.

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# RESPONSE OF DIFFERENT GENOTYPES OF WHEAT, RICE AND BLACK BEANS TO ANTHER, EMBRYO AND OTHER TISSUE CULTURES

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**Abstract**

The objective of the basic studies we have been conducting in our laboratory is to establish callus induction and *in vitro* plant regeneration protocols starting with several tissues of Guatemalan varieties of wheat (*Triticum aestivum* L.), rice (*Oryza sativa* L.) and especially black bean (*Phaseolus vulgaris* L.) in order to obtain disease resistance, earliness, and dwarf plants. Wheat anthers and immature embryos of varieties Patzun, Comalapa, Chocoyo, and Xequijel cultured in $N_6$, Potato II, and MS basal media supplemented with auxin and cytokinin gave the best responses in callus induction and plant regeneration. Anthers and mature embryos of *indica* rice varieties Precozicta and Virginia, when cultured in MS, $B_5$, $N_6$, and Potato II basal media with different hormonal combinations gave a good response in callus induction. However, a satisfactory response in plant regeneration was not obtained. With black beans, when hypocotyls and mature embryos of black bean varieties Quinack Che and Parramos were cultured in MS basal medium supplemented with different concentrations of NAA and kinetin, more than 60% callus induction was produced. When Quinack Che calli were transferred to MS basal medium supplemented with 1 mg/l NAA plus 0.5 mg/l BAP, green points of regeneration were visible in these calli.

## 1. INTRODUCTION

The potential for plant improvement through the culture of plant cells has been the subject of several studies [1-4]. Mutations and changes in ploidy can be induced in single cells by chemicals or radiation [5-7]. The environment of the treated cells can be managed so that desirable mutations will be selected through the survival of certain cells, the culture of those cells into callus tissue and from the callus the generation of plants with the desired characteristics [8,9]. If haploid cells are used, such as those derived from pollen, recessive characters are immediately visible and can be fixed in the resulting progeny. Plants produced through tissue culture often have natural changes in ploidy or express mutation [7]. These changes may be exploited as new sources of variation and used in conventional breeding programs.

In some species when pollen from $F_1$ or $F_2$ plants from conventional crosses is cultured, homozygosity, after diploidization, is normally obtained in one step with no further segregation. Where diploidization does not take place naturally, haploid plants produced through anther or ovary culture can be diploidized by treating the calli or developing plantlets with colchicine [10]. Sources of variation from a pure variety could be cryptic residual heterozygosity, chromosome mutation, and gene mutation. In pollen from $F_1$ plants there are these same sources of variation as well as recombination.

Wheat plants were obtained through anther culture in several laboratories in the early 1970's [11-14]. Pollen plant induction is now fairly easy and its frequency has been increased to such a level that wheat researchers have tried to apply the anther culture technique to breeding programmes. A winter wheat variety, Jinghua-1, which was developed by this approach is being released in northern China [15], and there have also been other valuable wheat lines obtained with this technique [16].

A number of methods have been employed in the production of pollen-derived embryos in rice. These include shedding culture [17], cold treatment, and preculture of anthers before isolation of pollen [18]. Green-plant regeneration is currently recognized as the most limiting step in the process. It has been greatly improved through incorporation of abscisic acid (ABA) in the regeneration medium [19]. Useful future applications of induced mutations include further development of photosensitive genetic male sterile mutants; and induction of dominant genetic male steriles, of cytoplasmic male steriles, of apomixis and of herbicide resistance. Induction of herbicide resistance through tissue culture has now been reported in many crops and continues to be of interest, especially to biotechnology firms. In rice, Oard and Rutger [20] sought imidazolinone resistant rice mutants, first through tissue culture and then through EMS mutagenized seed populations. At present, a selection from the mutagenized seed populations appears stable and is being studied genetically.

In legumes, such as *Phaseolus vulgaris* and *Phaseolus coccineus* the *in vitro* method has not been sufficiently developed, and the degree of success of this technique has still far to go. *In vitro* plant regeneration from bean leaf explants was reported first by Crocomo *et al.* [21]. Two plantlets were induced in a test of nine cultures. Organogenesis was observed by Sreedhar and Mehta [22] using hypocotyls and epicotyls of *Phaseolus lunatus* as explants. Allavena and Rossetti [23] and Allavena *et al.* [24] obtained plant regeneration by culturing immature cotyledons of *P. coccineus* variety Bianco di Spagna. Highly efficient plant regeneration has been achieved by Kumar *et al.* [25] by plating, on agar medium, long-term cell suspension cultures established from leaf-derived calli of tepary bean (*P. acutifolius).* Other authors [26, 27] recovered embryoids at different stages of development but few plants from tissue culture of *P. vulgaris*.

## 2. MATERIALS AND METHODS

### *2.1. Anther culture response of ten wheat varieties.*

Anthers with microspores in the uninucleate stage of the wheat varieties Patzun, Chocoyo, Comalapa, Chivito, Villa Laura, Zaragoza, Narino, Sara and the advanced line Mon's Imuri, were cultured in two basal media, N126 [28] or Potato II [29], supplemented with 2 mg/l 2,4 dichlorophenoxyacetic acid (2,4-D), 2 mg/l naphthalenacetic acid (NAA), 2 mg/l 2,4-D + 0.5 mg/l NAA or 2 mg/l NAA + 0.5 mg/l 2,4-D, 6% sucrose, adjusted to pH 5.7. Gerber jars with 20 ml of medium were used. Each one contained 60 anthers from one spike. Before being cultured, the anthers were cold-treated for 7 days at 6°C. Three hundred anthers were used per treatment.

### *2.2. Effect of gamma irradiation and basal media in the wheat variety Chocoyo.*

Seeds were equilibrated to 13% water moisture content, then irradiated with 0, 5, 15, and 25 krad (0, 50, 150 and 250 Gy) doses. Plants from the irradiated seeds were grown and the anthers were evaluated for callus induction in two basal media, $N_6$ and Potato II, supplemented with 2 mg/l 2,4-D and 9% sucrose in liquid media. Calli obtained were transferred to MS basal medium [30], which was supplemented with 6-benzilaminopurine (BAP), (1 or 2 mg/l), 3% sucrose and 0.8% agar for plant regeneration.

### *2.3. Evaluation of factors that affect callus induction and plant regeneration in the wheat varieties Chocoyo, Comalapa, and Patzun.*

The factors that affect callus formation and plant regeneration were evaluated in the wheat varieties Patzun, Comalapa, and Chocoyo. Two basal media, $N_6$ and Potato IV [31], were evaluated for all of the varieties. The hormonal combinations evaluated were : 2 mg/l 2,4-D, 2 mg/l NAA, 1.5

mg/l 2,4-D plus 0.5 mg/l kinetin, 1.5 mg/l NAA plus 0.5 mg/l kinetin, 2 mg 2,4-D plus 1 mg/l kinetin or 2 mg/l NAA plus 1 mg/l kinetin. The sucrose concentrations evaluated were 3%, 6%, and 9%. The effect of cold treatment before anther culture was evaluated for the variety Patzun. Anthers were treated for 0, 2, 4, 6, 8, and 10 days at 6°C. All media were adjusted to pH 5.7 and sterilized at 121°C and 15 psi for 15 minutes.

The wheat varieties were sown in the field, the uninucleate stage was determined and spikes with anthers at that stage were collected and placed in cold treatment at 6°C for seven days before culturing. Anthers used in the evaluation of the effect of cold treatment in the wheat variety Patzun were placed at 6°C for 0, 2, 4, 6, 8, and 10 days. After the cold treatment the spikes were sterilized for ten minutes in a solution of sodium hypochlorite at 0.5%, washed three times with distilled sterile water and placed in petri dishes. The anthers were then removed, placed in the callus induction media, and incubated at 28°C in the dark. After 35 days the number of anthers forming calli was determined and the calli were transferred to the basal media with 3% sucrose with or without growth regulators. Calli in these media were incubated at 25 +/- 3°C under a 24 hour photoperiod. Three weeks after transplanting, the number of regenerated plants was determined.

The evaluation of the factors that affect callus induction and plant regeneration in wheat was done under a randomized experimental design with a factorial structure. The experiments were different for each variety and factor. Seven replicates were used for each treatment, each replicate being a vial of 120 ml containing 15 ml of media and 60 anthers.

*2.4. Response of five wheat varieties to callus induction and plant regeneration.*

The wheat varieties used were Patzun, Chocoyo, Villa Laura, Xequijel, and Chivito. These varieties were cultured in $N_6$ and Potato II basal media to induce callus formation. The basal media were supplemented with 2 mg/l of NAA and 0.5 mg/l 2,4-D. The calli produced were transferred to a solid basal medium. This basal medium was the plant regeneration Murashige and Skoog (MS) supplemented with 1 and 2 mg/l IAA, and 0.5 benzyladenine (BA).

*2.5. Effect of gamma irradiation on callus induction and plant regeneration with immature embryo culture of three wheat varieties.*

Seeds of wheat varieties Comalapa, Patzun, and Chocoyo were treated with 0, 5, 8, 10, and 15 krad and sown in the field. Immature embryos were harvested and placed in Murashige and Skoog (MS) medium supplemented with 2% sucrose and 3 mg/l 2,4-D for callus induction. This same medium without hormones was used in plant regeneration.

*2.6. Response of wheat variety Patzun to immature embryo culture.*

Seeds of wheat variety Patzun were sown in the field and 20 days after pollination immature embryos were extracted and placed in basal media supplemented with 2% sucrose and auxins and cytokinins: 3 mg/l 2,4-D; 1.5 mg/l 2,4-D; 1.5 mg/l 2,4-D + 0.5 mg/l 6-benzylaminopurine (BAP); 3 mg/l 2,4-D + 1 mg/l BAP; 5 mg/l 2,4-D + 0.5 mg/l kinetin; 1 mg/l 2,4-D + 0.5 mg/l kinetin; 2.5 mg/l 2,4-D + 0.5 mg/l kinetin; 2 mg/l NAA + 0.5 mg/l kinetin; and 5 mg/l NAA + 0.5 mg/l kinetin.

*2.7. Response of four wheat varieties to gamma irradiation of anthers.*

Seeds of the wheat varieties Comalapa, Xequijel, Chocoyo, and Patzun were sown in the field. Spikes were harvested and irradiated with 0, 200, 500, 800, 1000, and 1500 rads (0, 2, 5, 8, 10, and

15 Gy). Comalapa and Patzun anthers were cultured in Potato IV basal medium supplemented with 1.5 mg/l NAA + 0.5 mg/l kinetin and 9% sucrose. Xequijel anthers were cultured in Potato IV basal medium supplemented with 1.5 mg/l 2,4-D + 0.5 mg/l kinetin and 9% sucrose. Chocoyo anthers were cultured in $N_6$ basal medium supplemented with 1.5 mg/l NAA + 0.5 mg/l kinetin + 9% sucrose.

*2.8. Response of five wheat varieties to callus induction and plant regeneration.*

Zaragosa, Comalapa, Narino-59, and Mon's Imuri were cultured. Callus induction media were $N_6$ and Potato II, each supplemented with 2 mg/l 2,4-D; 2 mg/l NAA; 2 mg/l 2,4-D + 0.5 mg/l NAA; 0.5 mg/l 2,4-D + 2 mg/l NAA, and one treatment without hormones. Plant regeneration medium used was MS supplemented with the following hormonal combinations: 2 mg/l BA; 1 mg/l BA + 0.5 mg/l IAA; 2 mg/l BA + 0.5 mg/l IAA; and one treatment without hormones.

*2.9. Effect of three basal media and different hormonal combinations on immature embryo culture of wheat varieties Xequijel and Chocoyo.*

Immature embryos of the wheat varieties Xequijel and Chocoyo were harvested from a greenhouse 12 days after self pollination and cultured in MS, Potato II, and $N_6$ basal medium. To each one of them was added 1 mg/l 2,4-D; 2 mg/l 2,4-D;1 mg/l 2,4-D plus 0.5 mg/l kinetin; 2 mg/l 2,4-D plus 0.5 mg/l kinetin; 1 mg/l NAA; 2 mg/l NAA; 2 mg/l NAA plus 0.5 mg/l kinetin; 1 mg/l NAA plus 0.5 mg/l kinetin. After 15 days--when calli were induced--they were transferred to MS basal medium without hormones or supplemented with 1 mg/l kinetin plus 0.5 mg/l IAA to regenerate plants.

*2.10. Anther culture response of the rice varieties Precozicta, Virginia, Precozicta x Colombia-1 and Virginia x Colombia-1.*

The rice plants were grown in the field. The uninucleate stage of the microspores was evaluated. Three basal media, $N_6$, $B_5$ (32), and Potato II were used. They were supplemented with 2 mg/l 2,4-D or 2 mg/l NAA. The sucrose concentration was 6% for the $N_6$ and Potato II basal media and 3% for the $B_5$ basal medium. The pH in all cases was 5.7. Gerber jars containing 20 ml of liquid medium were used for callus induction. Sixty anthers were placed in each jar. Prior to being placed in culture, panicles containing anthers in the uninucleate stage were maintained at 6°C for 7 days. To maintain moisture, they were wrapped in absorbent paper, moistened with distilled water, and placed in a plastic bag. Panicles were sterilized with a solution containing 20% bleach (1.05% sodium hypochlorite). The panicles were soaked in the solution for 10 minutes and then rinsed three times with distilled sterile water. Sterile panicles were dissected and the anthers were placed in culture. Three hundred anthers were used per treatment. Calli from the callus induction media were transferred to the plant regeneration medium, which contained the MS basal medium, supplemented with 1.5 or 2 mg/l BAP, 3% sucrose, and 0.8% agar, and adjusted to pH 5.7. Data were taken 50 days after inoculation for Precozicta and 36 days for ICTA Virginia.

*2.11. Effect of gamma irradiation on callus induction in the rice variety Precozicta.*

The effect of gamma rays on the anther culture response of the rice variety Precozicta was evaluated. Two basal media were used, $N_6$ and $B_5$. The $N_6$ basal medium was supplemented with 2 mg/l 2,4-D or 2 mg/l NAA. The $B_5$ basal medium was supplemented with 2 mg/l NAA. The radiation doses tested were 0, 5, 10, 15, and 20 krad. Radiation was applied to either the seed or the anthers collected from $M_1$ plants.

*2.12. Effect of gamma irradiation on callus induction and plant regeneration of hybrid rice.*

The effect of gamma radiation on callus induction and plant regeneration of the rice hybrids Precozicta x Colombia-1 and Virginia x Colombia-1 was evaluated. Seeds of the rice hybrids were equilibrated at 13% moisture and irradiated with 0, 10, 20, and 25 krad. After irradiation, the seeds were sown in the field. The uninucleate stage was determined, and panicles were collected and held at 6°C for seven days. Following the cold treatment, the panicles were sterilized for ten minutes in a solution containing 0.5% sodium hypochlorite, then washed three times with distilled sterile water. The anthers were dissected and placed in the callus induction medium, which consisted of $N_6$ basal medium supplemented with 2 mg/l 2,4-D and 6% sucrose. The anthers in the callus induction medium were incubated at 28°C in the dark. The number of anthers forming callus was determined 35 days after initiation of culture. Calli derived from the anthers were transferred to Murashige and Skoog (MS) basal medium with 3% sucrose supplemented either with 1.5 mg/l or 2 mg/l BAP, or without growth hormones and 0.8% agar.

*2.13. Response of the rice varieties Precozicta and Virginia to mature embryo and seedling root apex culture callus induction.*

Explants of mature embryos and seedling root apex were cultured in MS and $N_6$ basal media. Both were supplemented with 1, 2, and 5 mg/l 2,4-D; 1, 2 and 5 mg/l NAA; 1 mg/l 2,4-D + 0.5 mg/l kinetin; 1 mg/l 2,4-D + 1 mg/l kinetin; 2 mg/l 2,4-D + 0.5 mg/l kinetin; 2 mg/l 2,4-D + 1 mg/l kinetin; and 5 mg/l 2,4-D + 1 mg/l kinetin

*2.14. Effect of gamma irradiation on callus induction in hybrids of Virginia x Colombia-1 and reciprocals.*

The effect of gamma radiation on callus induction and plant regeneration on the rice hybrids Virginia x Colombia-1 and Colombia-1 x Virginia was evaluated. Radiation doses of 0, 5, 10, 15, and 20 krad were applied. Anthers were taken from plants developed from the irradiated seeds. The basal culture medium used was Potato II supplemented with 2 mg/l 2,4-D.

*2.15. Rice hybrid production.*

Hybrids Precozicta x Colombia-1 and Virginia x Colombia-1 were produced at the Bulbuxya Center for Tropical Agriculture in the southern part of Guatemala. The parent varieties were grown and the crosses made in the field. A total of 3779 seed were obtained in 1988

*2.16. Response of **Phaseolus vulgaris** variety Parramos to embryo, cotyledon, hypocotyl and seedling root apex culture callus induction.*

Explants of embryos, cotyledons, hypocotyls and seedling root apices of the variety Parramos were cultured in MS basal medium supplemented with the following hormonal doses: 1,3, and 5 mg/l 2,4-D; 1, 3, and 5 mg/l NAA; 2 mg/l 2,4-D + 1 mg/l NAA; 1 mg/l 2,4-D + 2 mg/l NAA; 3 mg/l 2,4-D + 2 mg/l Naa; and 2 mg/l 2,4-D + 3 mg/l NAA. Embryos were extracted from sterilized seeds and soaked for 24 hours. Root apices, hypocotyls and cotyledons were obtained after the seeds had been germinating for three days. The explants were placed in callus induction media.

*2.17. Response of **Phaseolus vulgaris** variety Quinack Che to embryo, hypocotyl and seedling root apex culture callus induction and regeneration.*

For callus induction, two basal media, MS and $B_5$, were used. MS basal media was supplemented with 0.5 mg/l NAA + 0.5 mg/l kinetin; 3 mg/l NAA + 0.5 mg/l kinetin; 5 mg/l NAA + 0.5 mg/l kinetin; 0.5 mg/l NAA + 3 mg/l kinetin; 3 mg/l NAA + 3 mg/l kinetin; and 5 mg/l NAA + 3 mg/l kinetin. $B_5$ basal media was supplemented with 1 mg/l 2,4-d + 0.5 mg/l kinetin; 0.5 mg/l 2,4-D + 0.5 mg/l kinetin; 1 mg/l 2,4-D + 0.5 mg/l kinetin; 0.1 mg/l 2,4-D + 1 mg/l kinetin; 0.5 mg/l 2,4-D + 1 mg/l kinetin; and 1 mg/l 2,4-D + 1 mg/l kinetin. After 15 days, callus was produced. Calli produced in MS basal medium were transferred to MS plant regeneration medium supplemented with 8 mg/l NAA + 0.5 mg/l kinetin.; 1 mg/l NAA + 0.5 mg/l BAP; 1.9 mg/l IAA + 1 mg/l NAA + 0.19 mg/l kinetin + boiled bean seed extract; and 8 mg/l IAA + 0.5 mg/l BAP + 0.1 mg/l gibberellic acid.

Calli produced in $B_5$ basal medium were transferred to the following regeneration media:

(a) RB (33) supplemented with 1 mg/l Picloram + 4 mg/l BA + 0.5 mg/l IBA.
(b) $B_5$ supplemented with 2 mg/l IAA + 1 mg/l BAP + 0.1 mg/l gibberellic acid.
(c) MS supplemented with 1.96 mg/l Picloram + 0.19 mg/l NAA + raw bean seed extract.
(d) $MS_5$ supplemented with 0.18 mg/l NAA + 2.25 mg/l BA.
(e) $MS_5$ supplemented with 2.25 mg/l BA.

Finally, calli with green points were transferred to two continuous cultures.

*2.18. Efficiency of the two methods in X, S, and Y virus elimination and response of potato (**Solanum tuberosum**) variety Loman to tuber induction.*

Potato variety Loman plants infected with X, S and Y viruses were collected in the field. Virus presence was detected with the enzyme-linked immunosorbent assay (ELISA) test. Slips and meristems were cultured in MS basal medium supplemented with 0.25 mg/l gibberellic acid, 2 m/l calcium pantothenate, 3% sucrose, 0.6% agar and 0.4 g/l thiamine hydrochloride. Five hundred mg/l chlorocholine chloride and 0, 2.5, 5, 7.5, and 10 mg/l doses of 6-benzylaminopurine were used for tuber induction. Thermotherapy was conducted in an oven with inside light (10 000 lux) at 37°C for 32 days (33).

## 3. RESULTS AND DISCUSSION

*3.1. Anther culture response of ten Guatemalan wheat varieties.*

The wheat varieties Chocoyo, Patzun, Comalapa. Villa Laura and Xequijel formed calli in the callus induction media. The best response was by Chocoyo. The other varieties gave a low response (Table I). Narino, Zaragoza, Chivito, Sara, and Mon's Imuri did not form callus in any media evaluated. Each treatment included 300 anthers.

*3.2. Effect of gamma irradiation and basal media on the wheat variety Chocoyo.*

Anthers derived from plants treated with 25 krad had a lower response to callus induction compared with anthers derived from non-treated plants or treated with 5 or 15 krad. The Potato II basal medium gave the most uniform response. Table II shows the effect of the gamma radiation and the basal media on callus induction in the wheat variety Chocoyo.

TABLE I. EFFECT OF TWO BASAL MEDIA AND FOUR HORMONAL COMBINATIONS ON THE INDUCTION OF CALLUS IN FIVE WHEAT VARIETIES.

| Variety | Basal medium | Hormonal combination | | Callus induction* (%) |
|---|---|---|---|---|
| | | 2,4-D (mg/l) | NAA (mg/l) | |
| Patzún | $N_6$ | 2.0 | 0.0 | 02 |
| Patzún | $N_6$ | 0.0 | 2.0 | 03 |
| Comalapa | $N_6$ | 0.0 | 2.0 | 01 |
| Comalapa | $N_6$ | 2.0 | 0.5 | 02 |
| Comalapa | $N_6$ | 0.5 | 2.0 | 04 |
| Comalapa | Potato II | 2.0 | 0.0 | 01 |
| Comalapa | Potato II | 0.0 | 2.0 | 02 |
| Comalapa | Potato II | 0.5 | 2.0 | 01 |
| Chocoyo | $N_6$ | 0.0 | 2-0 | 08 |
| Chocoyo | $N_6$ | 2.0 | 0.5 | 23 |
| Chocoyo | Potato II | 2.0 | 0.0 | 16 |
| Chocoyo | Potato II | 0.0 | 2.0 | 12 |
| Chocoyo | Potato II | 2.0 | 0.5 | 16 |
| Chocoyo | Potato II | 0.5 | 2.0 | 24 |
| Villa Laura | $N_6$ | 0.0 | 2.0 | 01 |
| Villa Laura | $N_6$ | 2.0 | 0.5 | 01 |
| Villa Laura | $N_6$ | 0.5 | 2.0 | 03 |
| Villa Laura | Potato II | 0.5 | 2-0 | 01 |
| Xequijel | Potato II | 0.5 | 2.0 | 01 |
| Xequijel | $N_6$ | 2.0 | 0.0 | 01 |
| Xequijel | $N_6$ | 0.0 | 2.0 | 01 |

*45 days after inoculation, Callus production = Calli produced (No.) x 100/Anthers plated (No.).

TABLE II. EFFECT OF BASAL MEDIA AND GAMMA IRRADIATION ON CALLUS PRODUCTION IN WHEAT VARIETY CHOCOYO.

| Basal medium | Radiation dose (krads) | Callus induction (%)* |
|---|---|---|
| $N_6$ | - | 9 |
| $N_6$ | 5 | 27 |
| $N_6$ | 15 | 2 |
| $N_6$ | 25 | 1 |
| Potato II | - | 10 |
| Potato II | 5 | 15 |
| Potato II | 15 | 16 |
| Potato II | 25 | - |

*See Table I.

*3.3. Evaluation of factors that affect callus induction and plant regeneration in the Guatemalan wheat varieties Chocoyo, Comalapa and Patzun.*

In Patzun wheat, the $N_6$ and Potato IV basal media supplemented with either 2 mg/l NAA or 2 mg/l 2,4-D gave the best callus induction; but the differences between basal media and hormonal

combinations were not significant (Table III). Sucrose concentration affected callus induction. Plant regeneration was obtained with 9% sucrose (Table IV). The cold treatment applied to anthers before culturing affected plant regeneration, but not callus induction. Untreated anthers or anthers treated for two days gave the higher number of regenerated plants. Table V presents the effect of cold treatment on callus induction and plant regeneration on the variety Patzun.

TABLE III. EFFECT OF TWO BASAL MEDIA AND SIX HORMONAL COMBINATIONS ON CALLUS INDUCTION IN THE WHEAT VARIETY PATZÚN.

| Basal medium | Hormonal combination | | | Callus induction (%) |
|---|---|---|---|---|
| | 2,4-D (mg/l) | NAA (mg/l) | Kinetin (mg/l) | |
| $N_6$ | 2.0 | 0.0 | 0.0 | 1.9 |
| $N_6$ | 0.0 | 2.0 | 0.0 | 1.9 |
| $N_6$ | 1.5 | 0.0 | 0.5 | 0.9 |
| $N_6$ | 0.0 | 1.5 | 0.5 | 1.4 |
| $N_6$ | 2.0 | 0.0 | 1.0 | 0.7 |
| $N_6$ | 0.0 | 2.0 | 1.0 | 0.7 |
| Potato IV | 2.0 | 0.0 | 0.0 | 1.4 |
| Potato IV | 0.0 | 2.0 | 0.0 | 1.2 |
| Potato IV | 1.5 | 0.0 | 0.5 | 0.2 |
| Potato IV | 0.0 | 1.5 | 0.5 | 0.5 |
| Potato IV | 2.0 | 0.0 | 1.0 | 0.0 |
| Potato IV | 0.0 | 2.0 | 1.0 | 0.2 |

TABLE IV. EFFECT OF BASAL MEDIA, HORMONAL COMBINATIONS AND SUCROSE CONCENTRATIONS ON CALLUS INDUCTION AND PLANT REGENERATION IN THE WHEAT VARIETY PATZÚN.*

| Basal medium | Hormonal combination | | | Sucrose concentration (%) | Callus induction (%) | Plant regeneration (%) |
|---|---|---|---|---|---|---|
| | 2,4-D (mg/l) | NAA (mg/l) | Kinetin (mg/l) | | | |
| $N_6$ | 2.0 | 0.0 | 0.0 | 3 | 0.5 | 0.0 |
| $N_6$ | 0.0 | 2.0 | 0.0 | 6 | 0.5 | 0.0 |
| $N_6$ | 2.0 | 0.0 | 0.0 | 6 | 1.4 | 0.0 |
| $N_6$ | 1.5 | 0.0 | 0.5 | 6 | 0.5 | 0.0 |
| $N_6$ | 2.0 | 0.0 | 0.0 | 9 | 1.9 | 0.0 |
| $N_6$ | 1.5 | 0.0 | 0.5 | 9 | 1.2 | 20.0 |
| Potato IV | 0.0 | 1.5 | 0.5 | 3 | 0.5 | 0.0 |
| Potato IV | 1.5 | 0.0 | 0.5 | 3 | 0.2 | 0.0 |
| Potato IV | 0.0 | 1.5 | 0.0 | 6 | 5.5 | 43.5 |
| Potato IV | 0.0 | 1.5 | 0.5 | 6 | 5.0 | 47.6 |
| Potato IV | 2.0 | 0.0 | 0.0 | 6 | 0.4 | 100.0 |
| Potato IV | 1.5 | 0.0 | 0.5 | 6 | 1.2 | 0.0 |
| Potato IV | 0.0 | 2.0 | 0.0 | 9 | 7.1 | 36.7 |
| Potato IV | 0.0 | 1.5 | 0.5 | 9 | 7.6 | 31.3 |
| Potato IV | 2.0 | 0-0 | 0.0 | 9 | 1.7 | 100.0 |
| Potato IV | 1.5 | 0.0 | 0.5 | 9 | 0.4 | 50.0 |

*Only treatments that induced callus are shown.

TABLE V. EFFECT OF COLD TREATMENT ON CALLUS INDUCTION AND PLANT REGENERATION IN THE WHEAT VARIETY PATZÚN.

| Cold treatment (days) | Callus induction (%) | Plant regeneration (%) |
|---|---|---|
| 0 | 1.7 | 33.3 |
| 2 | 1.8 | 31.2 |
| 4 | 0.8 | 0.0 |
| 6 | 0.9 | 1.3 |
| 8 | 1.1 | 0.1 |
| 10 | 0.6 | 0.0 |

With the wheat variety Comalapa, callus induction and plant regeneration were increased when the sucrose concentration was increased from 3% to 9%. The best results were obtained using Potato IV as the basal medium, supplemented with 2 mg/l NAA or 1.5 mg/l NAA plus 0.5 mg/l kinetin. Table VI shows the effect of basal media and sucrose concentrations on callus induction and plant regeneration on Comalapa wheat.

TABLE VI. EFFECT OF BASAL MEDIA, HORMONAL COMBINATIONS AND SUCROSE CONCENTRATIONS ON CALLUS INDUCTION AND PLANT REGENERATION IN THE WHEAT VARIETY COMALAPA.

| Basal medium | Hormonal combination | | Sucrose concentration (%) | Callus induction (%) | Plant regeneration (%) |
|---|---|---|---|---|---|
| | NAA (mg/l) | Kinetin (mg/l) | | | |
| $N_6$ | 2.0 | 0.0 | 3 | 0.0 | 0.0 |
| $N_6$ | 1.5 | 0.5 | 3 | 0.2 | 100.0 |
| $N_6$ | 2.0 | 0.0 | 6 | 1.2 | 0.0 |
| $N_6$ | 1.5 | 0.5 | 6 | 0.5 | 50.0 |
| $N_6$ | 2.0 | 0.0 | 9 | 2.6 | 9.1 |
| $N_6$ | 1.5 | 0.5 | 9 | 1.7 | 100.0 |
| Potato IV | 2.0 | 0.0 | 3 | 0.0 | 0.0 |
| Potato IV | 1.5 | 0.5 | 3 | 0.5 | 0.0 |
| Potato IV | 2.0 | 0.0 | 6 | 0.0 | 0.0 |
| Potato IV | 1.5 | 0.5 | 6 | 1.0 | 0.0 |
| Potato IV | 2.0 | 0.0 | 9 | 1.4 | 66.7 |
| Potato IV | 1.5 | 0.5 | 9 | 4.5 | 23.5 |

With the variety Chocoyo, there were no differences among basal media, hormonal combinations, or sucrose content in callus induction. The best callus induction (6.9%) was obtained with basal media $N_6$ supplemented with 1.5 mg/l NAA plus 0.5 mg/l kinetin and 9% sucrose.

*3.4. Response of five wheat varieties to callus induction and plant regeneration.*

Response of the wheat varieties Patzun, Chocoyo, Villa Laura, Xequijel, and Chivito was studied. With Chocoyo the Potato II basal medium supplemented with 2 mg/l NAA + 0.5 mg/l 2,4-D

gave the best callus induction. With Patzun and Xequijel, $N_6$ supplemented with 2 mg/l NAA resulted in the best callus induction. With Chocoyo, $N_6$ supplemented with 2 mg/l NAA + 0.5 mg/l of 2,4-D gave the highest percentage of callus induction (Table VII). The Chivito variety gave no response. Only Patzun responded in plant regeneration, with the best response being from the MS basal medium supplemented with 1 mg/l IAA and 0.5 mg/l BA, 66.6% green plants (Tables VIII-X).

TABLE VII. EFFECT OF BASAL MEDIA AND HORMONAL COMBINATIONS ON CALLUS INDUCTION IN THE WHEAT VARIETY CHOCOYO.

| Basal medium | Hormonal combination 2,4-D (mg/l) | NAA (mg/1) | Kinetin (mg/l) | Callus induction (%) |
|---|---|---|---|---|
| $N_6$ | 2.0 | 0.0 | 0.0 | 3.3 |
| $N_6$ | 0.0 | 2.0 | 0.0 | 1.0 |
| $N_6$ | 1.5 | 0.0 | 0.5 | 3.1 |
| $N_6$ | 0.0 | 1.5 | 0.5 | 1.2 |
| $N_6$ | 2.0 | 0.0 | 1.0 | 1.2 |
| $N_6$ | 0.0 | 2.0 | 1.0 | 2.6 |
| Potato IV | 2.0 | 0.0 | 0.0 | 0.0 |
| Potato IV | 0.0 | 2.0 | 0.0 | 1.4 |
| Potato IV | 1.5 | 0.0 | 0.5 | 0.2 |
| Potato IV | 0.0 | 1.5 | 0.5 | 0.0 |
| Potato IV | 2.0 | 0.0 | 1.0 | 0.2 |
| Potato IV | 0.0 | 2.0 | 1.0 | 0.0 |

TABLE VIII. EFFECT OF BASAL MEDIA, HORMONAL COMBINATIONS AND SUCROSE CONCENTRATIONS ON CALLUS INDUCTION IN THE WHEAT VARIETY CHOCOYO.

| Basal medium | Hormonal combination 2,4-D (mg/l) | NAA (mg/1) | Kinetin (mg/l) | Sucrose concentration (%) | Callus induction (%) |
|---|---|---|---|---|---|
| $N_6$ | 0.0 | 1.5 | 0.5 | 3 | 0.23 |
| $N_6$ | 1.5 | 0.0 | 0.5 | 3 | 2.62 |
| $N_6$ | 0.0 | 1.5 | 0.5 | 6 | 2.57 |
| $N_6$ | 1.5 | 0.0 | 0.5 | 6 | 2.85 |
| $N_6$ | 0.0 | 1.5 | 0.5 | 9 | 6.90 |
| $N_6$ | 1.5 | 0.0 | 0.5 | 9 | 0.70 |
| Potato IV | 0.0 | 1.5 | 0.5 | 9 | 0.95 |
| Potato IV | 1.5 | 0.0 | 0.5 | 9 | 0.23 |

*3.5. Effect of gamma radiation varieties on callus induction and plant regeneration with immature embryo culture of three wheat varieties.*

In the variety Comalapa, callus induction across all treatments ranged from 59 to 100 calli per 100 immature embryos cultured. The 8 krad dose gave the highest number of regenerated plants, 62%, and had 99% callus induction. With Patzun, callus induction across all treatments ranged from

78 to 100% across all treatments. The highest percentage of regenerated plants, 98%, was from the 15 krad treatment. For the Chocoyo variety, the range in callus induction across all treatments was 88 to 99%. Plant regeneration ranged from 34 to 48%, with the 15 krad dose resulting in the greatest number of plants. Data for the three varieties are presented in Table XI.

TABLE IX. EFFECT OF BASAL MEDIA AND HORMONAL COMBINATIONS ON CALLUS INDUCTION IN THE WHEAT VARIETIES PATZÚN, VILLA LAURA, CHOCOYO, AND XEQUIJEL.

| Variety | Basal medium | Hormonal combination 2,4-D (mg/l) | Hormonal combination NAA (mg/l) | Callus induction (%) |
|---|---|---|---|---|
| Patzún | $N_6$ | 0.0 | 2.0 | 3.0 |
| | $N_6$ | 2.0 | 0.0 | 2.3 |
| | $N_6$ | 0.5 | 2.0 | 1.0 |
| Villa Laura | $N_6$ | 0.5 | 2.0 | 3.0 |
| | Potato II | 0.5 | 2.0 | 1.3 |
| | $N_6$ | 2.0 | 0.0 | 1.0 |
| | $N_6$ | 0.0 | 2.0 | 1.0 |
| | $N_6$ | 2.0 | 0.5 | 1.0 |
| Chocoyo | Potato II | 0.5 | 2.0 | 24.3 |
| | $N_6$ | 2.0 | 0.5 | 23.3 |
| | Potato II | 2.0 | 0.0 | 16.0 |
| | Potato II | 2.0 | 0.5 | 16.0 |
| | Potato II | 0.0 | 2.0 | 11.0 |
| | $N_6$ | 0.0 | 2.0 | 8.3 |
| | $N_6$ | 0.0 | 0.0 | 0.3 |
| Xequijel | $N_6$ | 0.0 | 2.0 | 1.3 |
| | $N_6$ | 2.0 | 0.0 | 1.0 |
| | Potato II | 0.5 | 2.0 | 1.0 |
| | Potato II | 2.0 | 0.0 | 0.3 |
| | $N_6$ | 0.5 | 2.0 | 0.3 |

TABLE X. PLANT REGENERATION RESPONSE IN THE WHEAT VARIETY PATZÚN.

| Callus induction medium Basal | 2,4-D (mg/l) | NAA (mg/1) | Regeneration medium Basal | IAA (mg/l) | BA (mg/l) | Green plants (%) |
|---|---|---|---|---|---|---|
| $N_6$ | 0.0 | 2.0 | | 0.0 | 0.0 | 61.53 |
| $N_6$ | 2.0 | 0.0 | | 1.0 | 0.5 | 66.66 |

TABLE XI. CALLUS INDUCTION AND PLANT REGENERATION RESPONSE TO GAMMA IRRADIATION OF IMMATURE EMBRYO CULTURES IN MS BASAL MEDIUM SUPPLEMENTED WITH 2% SUCROSE AND 3 mg/l 2,4-D.

| Variety | Treatment (krads) | Callus induction (%) | Plant regeneration (%)* |
|---|---|---|---|
| Comalapa | 0 | 59 | 19 |
| | 5 | 60 | 11 |
| | 8 | 99 | 61 |
| | 10 | 100 | 41 |
| | 15 | 99 | 45 |
| Patzún | 0 | 98 | 43 |
| | 5 | 88 | 37 |
| | 8 | 100 | 31 |
| | 10 | 78 | 33 |
| | 15 | 98 | 118 |
| Chocoyo | 0 | 94 | 47 |
| | 5 | 87 | 34 |
| | 8 | 80 | 39 |
| | 10 | 99 | 47 |
| | 15 | 98 | 48 |

* In the plant regeneration phase the basal media MS without hormones was used.

### *3.6. Response of wheat variety Patzun to immature embryo culture.*

Callus induction ranged from 77 to 100% across all treatments for Patzun (Table XII). The highest percent induction, 100, was obtained with MS basal medium supplemented with 1.5 mg/l 2,4-D + 0.5 mg/l BAP or when the supplement was 5 mg/l 2,4-D + 0.5 mg/l kinetin. This latter treatment gave the highest number of regenerated plants, a total of 129.

TABLE XII. IMMATURE EMBRYO CULTURE RESPONSE OF PATZUN WHEAT TO DIFFERENT HORMONAL COMBINATIONS IN MS BASAL MEDIUM WITH 2% SUCROSE.

| Hormonal combination | | | | Callus | Plant |
|---|---|---|---|---|---|
| 2,4-D (mg/l) | BAP (mg/l) | Kinetin (mg/l) | NAA (mg/l) | induction (%) | regeneration (%) |
| 3.0 | 0.0 | 0.0 | 0.0 | 77 | 63 |
| 1.5 | 0.0 | 0.0 | 0.0 | 96 | 87 |
| 1.5 | 0.5 | 0.0 | 0.0 | 100 | 58 |
| 3.0 | 1.0 | 0.0 | 0.0 | 80 | 17 |
| 5.0 | 0.0 | 0.5 | 0.0 | 100 | 129 |
| 1.0 | 0.0 | 0.5 | 0.0 | 77 | 77 |
| 2.5 | 0.0 | 0.5 | 0.0 | 95 | 91 |
| 0.0 | 0.0 | 0.5 | 2.0 | 93 | 65 |
| 0.0 | 0.0 | 0.5 | 5.0 | 79 | 65 |

*3.7. Response of four wheat varieties to gamma irradiation of anthers.*

Only Patzun gave a response to irradiating the anthers, showing 20% callus induction with the 200 rad treatment. No response was obtained from the other varieties.

*3.8. Response of five wheat varieties to callus induction and plant regeneration.*

Of the five varieties in this evaluation, only Comalapa gave any response. Callus induction of 3.66% was obtained with $N_6$ basal medium supplemented with 0.5 mg/l 2,4-D + 2 mg/l NAA (Table XIII). Callus induction was 2.33% with Potato II basal medium supplemented with 2 mg/l NAA. When the calli induced in the $N_6$ basal medium were transferred to MS basal medium without hormones for plant regeneration, 76% green plants were produced (Table XIV). When calli developed in the Potato II basal medium were transferred to plant regeneration MS basal medium supplemented with 1 mg/l BA + 0.5 mg/l IAA, 66% green plants were produced.

TABLE XIII. RESPONSE OF COMALAPA WHEAT TO CALLUS INDUCTION BY ANTHER CULTURE.

| Basal medium | Induction medium Hormonal combination 2,4-D (mg/1) | NAA (mg/l) | Callus induction (%) |
|---|---|---|---|
| $N_6$ | 0.0 | 0.0 | 0.00 |
| $N_6$ | 2.0 | 0.0 | 0.33 |
| $N_6$ | 0.0 | 2.0 | 1.00 |
| $N_6$ | 2.0 | 0.5 | 1.33 |
| $N_6$ | 0.5 | 2.0 | 3.66 |
| Potato II | 0.0 | 0.0 | 0.33 |
| Potato II | 2.0 | 0.0 | 1.33 |
| Potato II | 0.0 | 2.0 | 2.33 |
| Potato II | 2.0 | 0.5 | 0.66 |
| Potato II | 0.5 | 2.0 | 1.00 |

TABLE XIV. RESPONSE OF COMALAPA WHEAT TO CALLUS INDUCTION AND PLANT REGENERATION BY ANTHER CULTURE.

| Callus induction medium | Regeneration medium | Plant regeneration (%) | Plants Green (%) | Albino (%) |
|---|---|---|---|---|
| $N_6$ + 0.5 mg/l 2,4-D + 2 mg/l NAA. | MS | 170 | 76.47 | 23.53 |
| Potato II + 2 mg/l NAA | MS + 1 mg/l BA + 0.5 mg/l IAA. | 60 | 66.66 | 33.33 |

*3.9. Effect of three basal media and different combinations of hormones on immature embryo culture of wheat varieties Xequilel and Chocoyo.*

For both varieties the best treatments were MS, Potato II, and $N_6$ basal media, each supplemented with 1 mg/l 2,4-D; 2 mg/l 2,4-D; and 2 mg/l 2,4-D plus 0.5 mg/l kinetin. All gave 100% callus induction (Table XV). Plantlets were not obtained in the plant regeneration phase, but when calli from the best callus induction treatments were transferred to MS basal medium, they produced a good number of green points, averaging 4 green regeneration points per callus (Table XVI).

TABLE XV. EFFECT OF MS, POTATO II, AND $N_6$ BASAL MEDIA AND DIFFERENT HORMONAL COMBINATIONS ON IMMATURE EMBRYO CULTURES OF XEQUIJEL AND CHOCOYO WHEAT.

| Hormonal combination | | | Callus induction (%) | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | Xequijel | | | Chocoyo | | |
| 2,4-D (mg/l) | NAA (mg/l) | Kinetin (mg/l) | MS | PII | $N_6$ | MS | PII | $N_6$ |
| 1.0 | 0.0 | 0.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 |
| 1.0 | 0.0 | 0.5 | 100.0 | 100.0 | 81.7 | 100.0 | 100.0 | 65.0 |
| 0.0 | 1.0 | 0.0 | 41.7 | 15.2 | 8.3 | 15.0 | 10.0 | 8.3 |
| 0.0 | 1.0 | 0.5 | 18.3 | 11.7 | 6.7 | 11.7 | 5.0 | 6.7 |
| 2.0 | 0.0 | 0.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 |
| 2.0 | 0.0 | 0.5 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 |
| 0.0 | 2.0 | 0.0 | 26.7 | 11.7 | 10.7 | 11.7 | 13.3 | 10.3 |
| 0.0 | 2.0 | 0.5 | 6.7 | 20.0 | 8.3 | 20.0 | 6.7 | 10.0 |

TABLE XVI. TREATMENTS THAT INDUCED LARGE NUMBERS OF GREEN POINTS IN CALLI OF XEQUIJEL AND CHOCOYO WHEAT DERIVED BY IMMATURE EMBRYO CULTURE.

| Hormonal combination for callus induction | | Basal medium for callus induction | | | | | |
|---|---|---|---|---|---|---|---|
| | | Xequijel | | | Chocoyo | | |
| 2,4-0 (mg/l) | Kinetin (mg/l) | MS | PII | $N_6$ | MS | PII | $N_6$ |
| 1.0 | 0.0 | x | x | x | x | x | x |
| 1.0 | 0.5 | x | x | x | x | x | x |
| 2.0 | 0.0 | x | x | x | x | x | x |
| 2.0 | 0.5 | x | x | x | x | x | x |

*3.10. Evaluation of the rice varieties Precozicta and Virginia and hybrids Precozicta x Colombia-1 and Virginia x Colombia-1.*

Precozicta and Virginia have a low callus induction response. For Precozicta, $N_6$ basal medium supplemented with 2 mg/l 2,4-D gave the highest percent of callus production (Table XVII). The highest percent callus induction for Virginia was with the $B_5$ basal medium supplemented either with 2 mg/l 2,4-D or 2 mg/l NAA. Although both varieties formed calli at a low rate, no plants were regenerated.

TABLE XVII. EFFECT OF THREE BASAL MEDIA AND TWO HORMONAL COMBINATIONS ON CALLUS INDUCTION WITH ANTHERS OF THE RICE VARIETIES PRECOZICTA AND VIRGINIA.

| Variety | Basal medium and hormonal combination | Callus induction (%)* |
|---|---|---|
| Precozicta | B5 + 2 mg/l 2,4-D | 3 |
| Precozicta | B5 + 2 mg/l NAA | 9 |
| Precozicta | $N_6$ + 2 mg/l 2,4-D | 19 |
| Precozicta | $N_6$ + 2 mg/l NAA | 2 |
| Precozicta | Potato II + 2 mg/l 2,4-D | - |
| Precozicta | Potato II + 2 mg/l NAA | 2 |
| Virginia | B5 + 2 mg/l 2,4-D | 10 |
| Virginia | B5 + 2 mg/l NAA | 12 |
| Virginia | $N_6$ + 2 mg/l 2,4-D | 4 |
| Virginia | $N_6$ + 2 mg/l NAA | - |
| Virginia | Potato II + 2 mg/l 2,4-D | 7 |
| Virginia | Potato II + 2 mg/l NAA | 2 |

*Callus production = Calli produced (No.) x 100/Anthers plated (No.).

Potato II basal media supplemented with 2 mg/l of 2,4-D gave the highest callus production for the Precozicta x Colombia-1 and Virginia x Colombia-1 hybrids (Table XVIII). A low response was obtained with $N_6$ as the basal medium or when the basal media were supplemented with 2 mg/l NAA.

TABLE XVIII. THE EFFECT OF CULTURE MEDIA ON CALLUS PRODUCTION IN RICE HYBRIDS.

| Hybrid | Basal medium and hormonal combination | Callus induction(%)* |
|---|---|---|
| Precozicta x Colombia-1 | N6 + 2 mg/l 2,4-D | 5 |
| Precozicta x Colombia-1 | N6 + 2 mg/l NAA | - |
| Precozicta x Colombia-1 | Potato II + 2 mg/l 2,4-D | 100 |
| Precozicta x Colombia-1 | Potato II + 2 mg/l NAA | 10 |
| Virginia x Colombia-1 | Potato II + 2 mg/l 2,4-D | 40 |
| Virginia x Colombia-1 | Potato II + 2 mg/l NAA | 3 |

*Data taken 37 days after plating.

### *3.11. Effect of gamma irradiation on callus induction in Precozicta rice.*

Gamma irradiation increased callus production when Precozicta was cultured on $N_6$ medium supplemented with 2 mg/l 2,4-D or 2 mg/l NAA. There was no trend in the effect of gamma radiation on callus induction (Table XIX). No plants were regenerated from calli.

### *3.12. Effect of gamma irradiation on callus induction and plant regeneration in rice hybrids.*

Gamma irradiation decreased callus induction in the rice hybrids Precozicta x Colombia-1 and Virginia x Colombia-1. The highest percent callus induction was from anthers collected from plants

derived from seeds that had not been irradiated. With both hybrids, as the irradiation dose increased, callus induction decreased (Table XX).

TABLE XIX. EFFECT OF GAMMA IRRADIATION AND CULTURE MEDIUM ON CALLUS PRODUCTION IN THE RICE VARIETY PRECOZICTA.

| Basal medium and hormonal combination | Radiation dose (krads) | Callus induction (%) |
|---|---|---|
| $N_6$ + 2 mg/l 2,4-D | 00 | 28 |
| $N_6$ + 2 mg/l 2,4-D | 05 | - |
| $N_6$ + 2 mg/l 2,4-D | 10 | 33 |
| $N_6$ + 2 mg/l 2,4-D | 15 | 7 |
| $N_6$ + 2 mg/l 2,4-D | 20 | 43 |
| $N_6$ + 2 mg/l NAA | 00 | 57 |
| $N_6$ + 2 mg/l NAA | 05 | 67 |
| $N_6$ + 2 mg/l NAA | 10 | 17 |
| $N_6$ + 2 mg/l NAA | 15 | - |
| $N_6$ + 2 mg/l NAA | 20 | 27 |
| B5 + 2 mg/l NAA | 00 | 21 |
| B5 + 2 mg/l NAA | 05 | 8 |
| B5 + 2 mg/l NAA | 10 | 4 |
| B5 + 2 mg/l NAA | 15 | - |
| B5 + 2 mg/l NAA | 20 | 6 |

TABLE XX. EFFECT OF GAMMA RADIATION ON CALLUS INDUCTION WITH ANTHERS OF THE RICE HYBRIDS PRECOZICTA X COLOMBIA-1 AND VIRGINIA X COLOMBIA-1.

| Hybrid | Radiation dose (krads) | Callus induction (%) |
|---|---|---|
| Precozicta x Colombia-1 | - | 17 |
| Precozicta x Colombia-1 | 10 | 14 |
| Precozicta x Colombia-1 | 20 | 11 |
| Precozicta x Colombia-1 | 25 | 3 |
| Virginia x Colombia-1 | - | 11 |
| Virginia x Colombia-1 | 10 | 4 |
| Virginia x Colombia-1 | 20 | 4 |
| Virginia x Colombia-1 | 25 | 2 |

*3.13. Response of the rice varieties Precozicta and Virginia to callus induction using mature embryos and root tips.*

With embryos of Precozicta, the highest callus induction, 39.6%, was on $N_6$ basal medium supplemented with 1 mg/l 2,4-D + 1 mg/l kinetin (Table XXI). With embryos cultured on MS basal medium, the highest callus induction, 33.0%, was on a medium supplemented with 2 mg/l 2,4-D + 1 mg/l kinetin.

TABLE XXI. RESPONSE OF PRECOZICTA AND VIRGINIA RICE TO CALLUS INDUCTION USING MATURE EMBRYO EXPLANTS.

| Explant | Basal medium | 2,4-D (mg/1) | Kinetin (mg/1) | NAA (mg/1) | Callus* induction (%) |
|---|---|---|---|---|---|
| Embryo Precozicta | MS | 1.0 | 0.5 | - | 19.8 |
| | | 1.0 | 1.0 | - | 9.9 |
| | | 2.0 | 0.5 | - | 13.2 |
| | | 2.0 | 1.0 | - | 33.0 |
| | | 5.0 | 0.5 | - | 9.9 |
| Embryo Precozicta | $N_6$ | 1.0 | 0.5 | - | 33.0 |
| | | 1.0 | 1.0 | - | 39.6 |
| | | 2.0 | 0.5 | - | 23.1 |
| | | 2.0 | 1.0 | - | 33.0 |
| | | 5.0 | 0.5 | - | 26.4 |
| | | 5.0 | 1.0 | - | 9.9 |
| Embryo Virginia | MS | 1.0 | - | - | 49.5 |
| | | 2.0 | - | - | 29.7 |
| | | 5.0 | - | - | 33.0 |
| | | 1.0 | 0.5 | - | 69.3 |
| | | 1.0 | 1.0 | - | 42.9 |
| | | 2.0 | 0.5 | - | 42.9 |
| | | 2.0 | 1.0 | - | 46.2 |
| | | 5.0 | 0.5 | - | 23.1 |
| | | 5.0 | 1.0 | - | 16.5 |
| | | - | - | 5.0 | 39.6 |
| Embryo Virginia | $N_6$ | 1.0 | - | - | 9.9 |
| | | 2.0 | - | - | 9.9 |
| | | 5.0 | - | - | 16.5 |
| | | 1.0 | 0.5 | - | 62.7 |
| | | 1.0 | 1.0 | - | 56.1 |
| | | 2.0 | 0.5 | - | 19.8 |
| | | 2.0 | 1.0 | - | 52.8 |
| | | 5.0 | 0.5 | - | 36.3 |
| | | 5.0 | 1.0 | - | 42.9 |
| | | - | - | 5.0 | 16.5 |

*Only treatments that gave response are shown.

The highest callus induction with embryos of Virginia, 69.3%, was on MS basal medium supplemented with 1 mg/l 2,4-D + 0.5 mg/l kinetin. On $N_6$ basal medium, the highest callus induction, 62.7%, was when the medium was supplemented with 1 mg/l 2,4-D + 0.5 mg/l kinetin.

With root tips of Precozicta cultured on MS basal medium supplemented with 2 mg/l NAA, there was 100% callus induction (Table XXII). On $N_6$ medium, a supplement of either 1 mg/l or 2 mg/l of NAA gave the highest percent callus induction, 85, for that medium. The root apices of Virginia failed to produce any callus.

TABLE XXII. RESPONSE OF PRECOZICTA RICE TO CALLUS INDUCTION USING ROOT APEX EXPLANTS.

| Explant | Basal medium | Hormonal combination 2,4-D (mg/1) | NAA (mg/l) | Kinetin (mg/l) | Callus induction (%) |
|---|---|---|---|---|---|
| Root apex Precozicta | MS | 1.0 | - | - | 59.4 |
| | | 2.0 | - | - | 95.7 |
| | | 5.0 | - | - | 66.0 |
| | | 5.0 | - | 1.0 | 82.5 |
| | | - | 1.0 | - | 79.2 |
| | | - | 2.0 | - | 10- |
| | | - | 5.0 | - | 59.4 |
| Root apex Precozicta | $N_6$ | 1.0 | - | - | 75.9 |
| | | 2.0 | - | - | 79.2 |
| | | 5.0 | - | - | 46.2 |
| | | - | 1.0 | - | 82.5 |
| | | - | 2.0 | - | 82.5 |
| | | - | 5.0 | - | 62.7 |

*3.14. Effect of gamma irradiation on callus induction in Virginia x Colombia-1 and reciprocal hybrids.*

When Virginia was the maternal parent of the hybrid, callus induction increased as the radiation dose increased, and anthers from plants developed from non-irradiated seed (control) produced no callus (Table XXIII). In contrast, when Colombia-1 was the maternal parent, callus induction was increased but was essentially the same regardless of the radiation treatment.

TABLE XXIII. GAMMA IRRADIATION AND MATERNAL EFFECT ON CALLUS PRODUCTION IN RICE HYBRIDS.

| Hybrid | Radiation dose (krads) | Callus induction (%) |
|---|---|---|
| Virginia x Colombia-1 | - | - |
| Virginia x Colombia-1 | 15 | 14 |
| Virginia x Colombia-1 | 20 | 26 |
| Colombia-1 x Virginia | - | 46 |
| Colombia-1 x Virginia | 15 | 48 |
| Colombia-1 x Virginia | 20 | 43 |

*3.15. Response of **Phaseolus vulgaris** variety Parramos to embryo, cotyledon, hypocotyl and root apex callus induction.*

The best response was obtained with MS basal medium supplemented with 2,4-D. In media supplemented only with NAA, only the root apices produced calli. Good results were obtained when

embryo explants were cultured in MS basal medium supplemented with 1 mg/l of 2,4-D. When hypocotyl explants were cultured in MS basal medium supplemented with 3 mg/l 2,4-D, 2 mg/l 2,4-D plus 1 mg/l NAA, or 1 mg/l 2,4-D plus 2 mg/l NAA high daily increases in dry weight were obtained (Table XXIV). The results indicate that hypocotyl explants are good sources for callus induction.

TABLE XXIV. DRY WEIGHT INCREMENT IN CALLUS PRODUCTION FROM EMBRYO, COTYLEDON, HYPOCOTYL, AND ROOT APEX EXPLANTS OF *PHASEOLUS VULGARIS* CV. PARRAMOS.

| Hormonal combination | | | Dry weight increment, mg | | | Daily |
|---|---|---|---|---|---|---|
| 2,4-D (mg/l) | NAA (mg/l) | Explant | 5 days | 10 days | 15 days | increment (mg) |
| 3.0 | - | Cotyledon | 10.9 | 3.3 | 0.2 | 0.96 |
| 3.0 | - | Hypocotyl | 5.5 | 16.5 | 19.1 | 2.74 |
| 2.0 | 1.0 | Hypocotyl | 15.7 | 5.5 | 12.8 | 2.26 |
| 1.0 | - | Embryo | 1.5 | 16.8 | 16.5 | 2.32 |
| 1.0 | 2.0 | Hypocotyl | 7.1 | 10.2 | 15.5 | 2.18 |
| 3.0 | - | Embryo | 1.7 | 8.4 | 16.9 | 1.70 |
| 2.0 | 1.0 | Embryo | 1.4 | 10.1 | 11.0 | 1.50 |
| 2.0 | 1.0 | Root | 3.3 | 9.9 | 11.0 | 1.20 |
| 1.0 | 2.0 | Root | 1.6 | 3.3 | 6.7 | 0.80 |
| 3.0 | - | Root | 9.2 | 6.8 | 2.0 | 0.80 |

*3.16. Response of* ***Phaseolus vulgaris*** *variety Quinack Che to mature embryo, hypocotyl, and seedling root apex callus induction and plant regeneration.*

When hypocotyl explants were cultured in MS basal medium supplemented with 5 mg/l NAA + 0.5 mg/l kinetin, callus induction was 100% (Table XXV). The heaviest fresh weight of callus was produced by the hypocotyl and embryo. When root explants were cultured in $B_5$ basal medium supplemented with 0.1 mg/l 2,4-D + 0.5 mg/l kinetin, or 0.5 mg/l 2,4-D + 0.5 mg/l kinetin., callus induction was 100% (Table XXVI).

In regard to plant regeneration, green points of regeneration were observed in calli of embryo explants cultured in MS basal medium and transferred to MS regeneration medium + 1 mg/l NAA + 0.5 mg/l BAP. Other treatments did not show any response. No plants were regenerated.

*3.17. Efficiency of the two methods in X, S and Y virus elimination and response of potato (****Solanum tuberosum****) variety Loman to tuber induction.*

High efficiency in X, S and Y virus elimination by thermotherapy was established. Difficulty in eliminating X and Y viruses by meristem culture was experienced (Table XXVII).

The best response in tuber production was induced by adding 10 mg/l BAP to the basal medium. Also, the largest tuber weight was obtained when 10 mg/l BAP were added to the basal medium (Table XXVIII).

TABLE XXV. RESPONSE OF *PHASEOLUS VULGARIS* CV. QUINACK CHE TO EMBRYO, HYPOCOTYL AND ROOT APEX CALLUS INDUCTION USING VARIOUS HORMONAL COMBINATIONS WITH MS BASAL MEDIUM.

| Hormonal combination | | | |
|---|---|---|---|
| NAA (mg/l) | Kinetin (mg/l) | Explant source | Callus induction (%) |
| 5.0 | 0.5 | Hypocotyl | 100.0 |
| 0.5 | 0.5 | Root | 97.9 |
| 0.5 | 3.0 | Root | 97.9 |
| 5.0 | 3.0 | Root | 97.9 |
| 3.0 | 0.5 | Hypocotyl | 97.9 |
| 3.0 | 3.0 | Hypocotyl | 97.9 |
| 5.0 | 3.0 | Hypocotyl | 97.9 |
| 5.0 | 0.5 | Embryo | 95.8 |
| 5.0 | 3.0 | Embryo | 95.8 |
| 3.0 | 0.5 | Root | 95.8 |
| 3.0 | 3.0 | Root | 95.8 |
| 0.5 | 3.0 | Hypocotyl | 95.8 |
| 0.5 | 0.5 | Hypocotyl | 95.5 |
| 3.0 | 3.0 | Embryo | 93.4 |
| 5.0 | 0.5 | Root | 90.5 |
| 3.0 | 0.5 | Embryo | 82.3 |
| 0.5 | 3.0 | Embryo | 81.8 |
| 0.5 | 0.5 | Embryo | 64.3 |

TABLE XXVI. RESPONSE OF *PHASEOLUS VULGARIS* CV. QUINACK CHE TO EMBRYO, HYPOCOTYL AND ROOT APEX CALLUS INDUCTION USING VARIOUS HORMONAL COMBINATIONS WITH $B_5$ BASAL MEDIUM.

| Hormonal combination | | | |
|---|---|---|---|
| 2,4-D (mg/l) | Kinetin (mg/l) | Explant source | Callus induction (%) |
| 0.1 | 0.5 | Root | 100.0 |
| 0.5 | 0.5 | Root | 100.0 |
| 0.1 | 0.5 | Embryo | 98.3 |
| 0.5 | 0.5 | Hypocotyl | 98.3 |
| 0.5 | 0.5 | Embryo | 96.5 |
| 1.0 | 1.0 | Embryo | 96.5 |
| 1.0 | 0.5 | Hypocotyl | 96.5 |
| 0.1 | 1.0 | Root | 94.6 |
| 1.0 | 0.5 | Embryo | 92.9 |
| 0.1 | 0.5 | Hypocotyl | 92.9 |
| 0.5 | 1.0 | Hypocotyl | 90.7 |
| 1.0 | 0.5 | Root | 86.5 |
| 0.1 | 1.0 | Root | 85.1 |
| 0.5 | 1.0 | Embryo | 85.1 |
| 0.5 | 1.0 | Root | 84.8 |
| 0.1 | 1.0 | Embryo | 84.4 |
| 1.0 | 1.0 | Root | 84.0 |
| 1.0 | 1.0 | Hypocotyl | 50.2 |

TABLE XXVII. EFFICIENCY OF THERMOTHERAPY AND MERISTEM CULTURE METHODS IN ELIMINATION OF X, S AND Y VIRUSES IN POTATO CV. LOMAN.

| Virus | Plants | Method | |
|---|---|---|---|
| | | Thermotherapy (% plants) | Meristem culture (% plants) |
| X | Healthy | 75.0 | 50.0 |
| | Infected | 04.0 | 50.0 |
| S | Healthy | 100.0 | 75.0 |
| | Infected | - | 25.0 |
| Y | Healthy | 87.5 | 62.5 |
| | Infected | 12.5 | 37.5 |

TABLE XXVIII. RESPONSE OF POTATO CV. LOMAN TO TUBER INDUCTION ON MS BASAL MEDIUM AT DIFFERENT DOSES OF BAP.

| BAP concentration (mg/l) | Mean No. tubers induced | Mean wt./tuber (mg) |
|---|---|---|
| 0.0 | 3 | 100 |
| 2.5 | 4 | 475 |
| 5.0 | 4 | 251 |
| 7.5 | 7 | 149 |
| 10.0 | 8 | 182 |

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# MUTATION BREEDING OF WHEAT IN S. PAULO STATE, BRAZIL

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**Abstract**

A mutation breeding program was initiated in 1985 at IAC in S. Paulo State, Brazil. Seeds from several cultivars were irradiated for selection of mutants for plant height, resistance to diseases, and aluminum toxicity tolerance. In another case, $F_4$ seeds from a cross between *T. aestivum L.* x *T. durum L.* were irradiated to increase genetic recombinations. Several genotypes from the different populations were selected and the first yield trial was conducted. In the case of the cultivars IAC-17, IAC-18 and IAC-24, the results indicated that genotypes with a semidwarf plant type and with an increase in resistance to diseases were obtained. Due to the occurrence of a frost, selected lines from Anahuac that are apparently tolerant to $Al^{+3}$ toxicity were killed, and seed is being multiplied for further evaluation in yield trials. Results from the first yield trials showed that some lines selected from the irradiated $F_4$ cross showed a high yield potential, with a combination of excellent agronomic characteristics. As the yield trials were conducted for one year only, the usefulness of the selected genotypes will be verified only after obtaining the results of the trials planned for additional years.

## 1. INTRODUCTION

The IAC wheat breeding programme began releasing improved cultivars of wheat to farmers in 1970. Because of the nature of the research described here, it is useful to review the IAC wheat breeding programme and to describe some of the cultivars that have been developed in the programme.

The cultivars IAC-17 and IAC-18 were recommended for growing on several types of soils [1] because they possess tolerance to $Al^{3+}$ toxicity, which limits the yield of susceptible cultivars in certain regions of the S.Paulo State. These two cultivars have high yield potential but they are tall, and in addition, they have become susceptible to new races of stem and leaf rust. More recent cultivars, such as IAC-24 [2], have a semidwarf plant type but are susceptible to *Helminthosporium* sp. and to leaf rust.

Some cultivars introduced from Mexico, including Anahuac, were developed on alkaline soils. In S. Paulo State they were recommended only for soils where aluminum was not a limiting factor because of the high susceptibility to aluminium toxicity exhibited by these genotypes. At the end of the 1980 decade, the cultivar Anahuac occupied the major wheat growing area (with and without irrigation) in S. Paulo State on soils free of aluminum.

There are two principal commercial types of wheat. One type is bread wheat, *(Triticum aestivum* L.), a hexaploid with 3 genomes (A, B and D), which represents the totality of Brazilian wheat production.The other type is durum wheat, *T. durum* L., a tetraploid with 2 genomes (A and B), which is used for macaroni (pasta). Brazil does not produce or import durum wheat, and *T. aestivum* is used to produce macaroni even though it is not suitable for such use. Because the durum wheat cultivars do not possess the D genome, which is the genome in which the genes for tolerance to aluminum are located, they are susceptible to acid soils and on such soils are low yielding. At

IAC, crosses are being made between *T. aestivum* L. and *T. durum* L. cultivars to increase genetic variability in order to select lines with high yield potential, semidwarf plant type, and better processing qualities for bread, macaroni and biscuits.

Several wheat mutation breeding projects were conducted in Brazil before 1970 [3]. Using gamma ray treatments, a mutant was selected that was resistant to stem rust. The mutant was used in the development of the cultivar IAS 63. Other research projects were conducted in several Brazilian States. In S.Paulo, some semidwarf and rust resistant mutants were obtained in a cooperative project between IAC and CENA [4-6].

Based on the above experiences, a mutation breeding programme involving IAC and CENA was started in 1985. The objective was to utilize gamma rays to obtain mutants for the following agronomic characteristics:

a) In IAC-17 and IAC-18 cultivars: semidwarf plant, resistance to leaf rust and *Helminthosporium.*
b) In IAC-24 cultivar: resistance to leaf rust and *Helminthosporium.*
c) In Anahuac cultivar: tolerance to aluminum toxicity.
d) In the hybrid population from the cross between BH 1146 (*T. aestivum* L.) and WIN'S" x AA"S" (*T. durum* L.): increase recombination in lines with high yield potential, semidwarf plant type,tolerance to aluminum, resistance to diseases, and better processing qualities.

## 2. MATERIALS AND METHODS

The programme utilized seeds of *T. aestivum* L. of the cultivars IAC-17, IAC-18, IAC-24 and Anahuac, and from the hybrid population ($F_4$ generation) of the cross BH 1146 (*T. aestivum* L.) and the line WIN"S" x AA"S" (*T. durum L.*). The gamma ray treatments were applied using the $^{60}Co$ source from CENA/USP in Piracicaba, S.P.

The sensitivity of the different materials was evaluated by irradiating seeds (12% moisture content) with the doses: 10, 15, 20, 25, 30, 35, 40, 45, and 50 krad, at a dose rate of 129 krad/hour. The doses for the treatments were selected on the basis of seedling height, survival, and development of the $M_1$ plants. Following the treatment with the selected doses, selection was initiated in the $M_2$ generation ($M_3$ in case of the Anahuac) under field conditions, evaluating the plants for height, cycle, resistance to diseases, and tolerance to aluminum toxicity.

Plant height was measured at maturity, from soil surface to the tip of the spike, excluding the awns. The plant cycle was based on the number of days from seedling emergence to flowering or to maturity. In the latter case, the genotypes were classified as early (100-120 days), normal (121-130 days), or late (more than 130 days). The Cobb modified method was used for leaf and stem rust evaluations [7].

Mildew (*Erisiphe graminis* ssp. *graminis*) and leaf spots *(Helminthosporium aestivum)* were evaluated [8] according to the following scoring system: 1 to 5% = resistant; 6 to 25% = moderately resistant; 26 to 50% = susceptible; and 51 to 99% = highly susceptible. Lodging was based on the percentage of plants that were lodged at maturity. Frost damage was based on the percentage of spikes that were affected at maturity. Selection for tolerance to aluminum was first conducted under field conditions at Itararé, where the soil normally contains a high concentration of $Al^{3+}$. To confirm the

tolerance, the selected lines were evaluated in nutrient solution [9], and plants able to grow at 6 ppm were considered as tolerant.

The selected plants from $M_2$ (or $M_3$, in case of Anahuac) were harvested individually (one spike per plant). Lines from selected plants of the $M_3$ and $M_4$ generations were observed under field conditions in comparison with the controls, and the best lines were selected to be evaluated in the $M_5$ generation. In this case, two or three lines from each genotype were analyzed. For Anahuac, the $M_6$ generation was evaluated in the same way at Itararé. The $M_6$ selected genotypes from IAC-17, IAC-18, IAC-24 and from the cross BHI146 x (WIN'S" x AA"S") were initially evaluated in advanced yield trials initiated in 1990. Due to frost, the trial that included Anahuac was lost and will be repeated.

A randomized block design with 3 replications was used in the field trials. The plot size was 6 rows x 3 m, with a 0.20 m spacing between rows, and the seeding rate was 80 seeds/ m. The trials were conducted at four locations: Campinas, Tatui, and Monte Alegre do Sul with sprinkler irrigation, and Maracai under upland conditions. Three genotypes selected from IAC-17 and the nine from IAC-18 were evaluated at Maracai. The genotypes from IAC-24 were tested at Monte Alegre do Sul and Tatui.

In regard to the cross BH 1146 x (WIN'S" x AA"S"), the genotypes were divided and the following trials were carried out:

Trial 1: 12 lines, Monte Alégre do Sul, Tatui
" 2: 23 " Campinas, Maracai
" 3: 22 " Campinas, Maracai
" 4: 22 " Campinas, Tatui
" 5: 23 " Campinas, Tatui
" 6: 21 " Monte Alégre do Sul, Tatui

## 3. RESULTS AND DISCUSSION

*Determination of gamma ray sensitivity*

Table 1 shows the seedling heights for Anahuac, IAC-17, IAC-18 and IAC-24 at 20 days after sowing, relative to the controls, which are considered as 100. As expected, the height decreased in all the populations as the doses increased. At the 20 krad dose, differences in sensitivity between the four cultivars were observed. Anahuac was the most sensitive and IAC-17, the least. Due to problems in germination, the results of the $F_4$ generation of the cross are not presented. The gamma ray doses were selected on the basis of the results presented in Table I and visual observation of the $M_1$ seedlings from the cross. The doses were 26 and 31 krads for Anahuac; 30 an 35 for IAC-17; 35 and 40 for IAC-18; and 25 and 30 krads for IAC-24. For the $F_6$ of the cross BH 1146 x (WIN'S" x AA"S"), the dose was 27.5 krad.

*The $M_1$ and $M_2$ generation*

Seeds were irradiated with the selected doses. Table II shows the number of irradiated seeds and the number of $M_2$ seeds harvested, using the bulk method. Table II also shows the number of $M_2$ seeds utilized to obtain the $M_2$ generation. In some cases only the highest dose was utilized.

TABLE I. EFFECT OF GAMMA RAYS IRRADIATION ON $M_1$ SEEDLING HEIGHT OF FOUR WHEAT CULTIVARS.

| Dose (krad) | Cultivars | | | |
|---|---|---|---|---|
| | Anahuac | IAC-17 | IAC-18 | IAC-24 |
| 0* | 100.0 | 100.0 | 100.0 | 100.0 |
| 10 | 98.7 | 103.0 | 91.2 | 106.0 |
| 15 | 99.6 | 105.8 | 92.0 | 106.0 |
| 20 | 89.9 | 107.8 | 89.2 | 100.8 |
| 25 | 82.4 | 99.4 | 78.0 | 88.6 |
| 30 | 69.3 | 94.9 | 70.5 | 85.2 |
| 35 | 39.9 | 80.8 | 63.0 | 67.9 |
| 40 | 31.7 | 65.9 | 45.5 | 60.6 |
| 45 | 23.5 | 48.6 | 23.3 | 43.4 |
| 50 | 12.6 | 37.8 | 19.6 | 33.5 |

*The seedling height of the controls, 20 days after planting, was considered as 100%.

TABLE II. RADIATION DOSES APPLIED AND $M_1$ AND $M_2$ POPULATIONS OF FIVE VARIETIES AND HYBRIDS.

| Materials | Doses (krad) | No. of seeds treated to obtain $M_1$ generation | No. of $M_2$ seeds harvested | No. of $M_2$ seeds planted |
|---|---|---|---|---|
| Anahuac | 26 | 163 000 | 60 000 | 60 000 |
| | 31 | 163 000 | 44 000 | 44 000 |
| IAC-17 | 30 | 170 000 | 1 240 000 | - |
| | 35 | 170 000 | 1 130 000 | 340 000 |
| IAC-18 | 35 | 160 000 | 1 250 000 | - |
| | 40 | 160 000 | 1 075 000 | 320 000 |
| IAC-24 | 25 | 150 000 | 105 000 | 105 000 |
| | 30 | 150 000 | 78 000 | 78 000 |
| Hybrid: | | | | |
| BH1146 x WIN'S" x AA"S" | 27.5 | 13 000 | 500 000 | 500 000 |

*Results from selection in the $M_2$, $M_3$, $M_4$, and $M_5$ generations*

As described earlier, after selection of individual plants in the $M_2$ generation ($M_3$ for Anahuac), the $M_3$, $M_4$ and $M_5$ progenies were evaluated under field conditions. The results are presented in Table 3. For Anahuac, 242 genotypes were selected in the $M_3$ generation under field conditions. These genotypes were evaluated in nutrient solution and the tolerant plants multiplied in the screenhouse for further evaluation. Due to the frost occurrence in 1990, spikes were available only from the surviving plants and these will be multiplied for yield trials in 1992.

*Advanced yield trial, including genotypes selected from IAC-17 cultivar*

As presented in Table III, after completing all selection steps, three mutant lines were chosen for the advanced yield trial, starting in 1990. Results of the trial conducted at Maracai are presented in Table IV.

TABLE III. NUMBER OF MUTANTS SELECTED AND EVALUATED IN THE $M_2$ THROUGH $M_6$ GENERATIONS FROM FOUR POPULATIONS.

| Materials | No. $M_3$ lines evaluated in field (1987) | No. $M_4$ lines evaluated in field (1988) | No. $M_5$ lines evaluated in field: preliminary yield trial (1989) | No. $M_6$ lines selected for advanced yield trials (1990) |
|---|---|---|---|---|
| Cultivars | | | | |
| IAC-17 | 259 | 197 | 90 | 3 |
| IAC-18 | 488 | 134 | 40 | 9 |
| IAC-24 | 483 | 159 | 26 | 8 |
| Hybrid: | | | | |
| BH1146 x (WIN"S"xAA"S") | 1604 | 2163 | 398 | 124 |

TABLE IV. ADVANCED TRIAL OF LINES SELECTED FROM IAC-17, MARACAI, 1990.

| Genotype | Cycle | Lodging[a] | *Helminthosporium*[b] | Rust[c] | | Mildew[b] | Plant height (cm) | $Al^{3+}$ [d] tolerance (6 ppm) | Yield (kg/ha) |
|---|---|---|---|---|---|---|---|---|---|
| | | | | Leaf | Stem | | | | |
| IAC-17 Control | Early | 2 | 20 | 5S | 5S | 10 | 102 | T | 1493 |
| IAC-17-3 | Early | 1 | 40 | 0 | 10S | 0 | 90 | T | 974 |
| IAC-17-4 | Early | 1 | 40 | 20S | 10S | 0 | 95 | T | 1685 |
| IAC-17-5 | Early | 0 | 40 | 10S | 10S | 0 | 85 | T | 1858 |
| L.S.D. (5% level) | | | | | | | | | 423 |

[a] % of plants lodging at maturity: 0=none, 1=1-20%, 2=21-40%, 3=41-60%, 4=61-80%, 5=81-100%.

[b] % of infected area, 0=immune, 1-5%=resistant, 6-25%=moderately resistant, 26-50%=susceptible, 51-99%=highly susceptible.

[c] Rust, leaf and stem--score shows % of infected area and reaction type: S=susceptible, MS=moderately susceptible, M=intermediate, MR=moderately resistant, R=resistant, t=trace.

[d] T=tolerant, S=susceptible.

All selected genotypes retained the plant cycle and aluminum tolerance (6 ppm) of the control. All showed a reduction in plant height, resulting in less lodging, and lower mildew symptoms than the control. However, there was an increase in *Helminthosporium* and rust symptoms, with the exception of IAC-17-3, which was free of leaf rust.

IAC-17-3 was lower yielding, whereas IAC-17-5 showed a high yield potential, although the yield value was not statistically significant. IAC-17-5 has an interesting semidwarf plant type and will be carefully observed in the 1991 and 1992 yield trials.

*Advanced yield trial including genotypes selected from IAC-18 cultivar*

Nine genotypes from IAC-18 were selected to be evaluated. Table V shows the result of the first advanced yield trial, conducted in Maracai. All lines showed a reduction in plant height as compared with the control, resulting in less lodging. With the exception of line IAC-18-12, the genotypes retained the same plant cycle and aluminum tolerance as the control. The lines had a lower incidence of rust and *Helminthosporium* but an increase in mildew. Some lines yielded the same as or less than the control, but IAC-187-15 and IAC-18-16 were higher yielding than the control. They are of special interest because they possess a combination of good agronomic traits that include high yield potential, a semidwarf plant type, and a lower incidence of *Helminthosporium* and rust. In addition, both retained the plant cycle and aluminum tolerance of the original variety. The only negative point was a small increase in mildew incidence.

TABLE V. ADVANCED TRIAL OF LINES SELECTED FROM IAC-18, MARACAI, 1990.

| Genotype | Cycle | Lodging[a] | *Helminthosporium*[a] | Rust[a] | | Mildew[a] | Plant height (cm) | $Al^{3+}$ [a] tolerance (6 ppm) | Yield (kg/ha) |
|---|---|---|---|---|---|---|---|---|---|
| | | | | Leaf | Stem | | | | |
| IAC-18 Control | Early | 2 | 30 | 20S | 20S | 0 | 90 | T | 1408 |
| IAC-18-8 | Early | 1 | 40 | 0 | 10S | 40 | 85 | T | 585 |
| IAC-18-9 | Early | 0 | 30 | 0 | 20S | 40 | 80 | T | 1051 |
| IAC-18-10 | Early | 0 | 40 | 10S | 10S | 40 | 70 | T | 1482 |
| IAC-18-11 | Early | 1 | 40 | 5S | 10S | 50 | 86 | T | 1437 |
| IAC-18-12 | Early | 0 | 20 | 0 | 0 | 40 | 85 | S | 1792 |
| IAC-18-13 | Early | 0 | 20 | 5S | 0 | 40 | 75 | T | 1684 |
| IAC-18-14 | Early | 0 | 10 | 10S | 5S | 20 | 75 | T | 1715 |
| IAC-18-15 | Early | 0 | 20 | 10S | 0 | 30 | 66 | T | 1842 |
| IAC-18-16 | Early | 0 | 20 | 0 | 5S | 10 | 76 | T | 1848 |

[a] For legends see Table IV. L.S.D. (5% level) 403

*Advanced yield trial including genotypes selected from IAC-24 cultivar*

Tables VI and VII present the results of yield trials conducted at Tatui and Monte Alegre do Sul that included 8 selected lines from IAC-24 and the control. For some characters, there were location differences. *Helminthosporium* occurred at both locations, and there was a general tendency for the mutants to have a lower incidence of the disease than the controls. With two exceptions, the same reaction was experienced with mildew. Rust occurred only at Tatui and the lines were more resistant than the controls. Only two genotypes failed to retain the aluminum tolerance of the original cultivar. The growth cycle and plant height of the mutants varied by location. At both locations IAC-24-L-15 was shorter than the control. At Tatui IAC-24-L16, IAC-24-L18, and IAC-24-L24 produced higher yields than the control but the differences were not statistically significant.

TABLE VI. ADVANCED TRIAL OF LINES SELECTED FROM IAC-24, TATUI, 1990.

| Genotype | Cycle[a] | Lodging[b] | *Helminthosporium*[b] | Leaf[b] rust | Frost[c] | Mildew[b] | Plant height (cm) | $Al^{3+}$ [b] tolerance (6 ppm) | Yield (kg/ha) |
|---|---|---|---|---|---|---|---|---|---|
| IAC-24 Control | 70 | 0 | 60 | 10S | 20 | 40 | 100 | T | 2791 |
| IAC-24-L15 | 85 | 0 | 20 | tS | 0 | 0 | 87 | T | 2621 |
| IAC-24-L16 | 85 | 0 | 50 | 0 | 40 | 20 | 100 | T | 3560 |
| IAC-24-L17 | 80 | 0 | 40 | 0 | 20 | 0 | 85 | S | 2625 |
| IAC-24-L18 | 74 | 0 | 40 | tS | 0 | 10 | 100 | S | 3273 |
| IAC-24-L19 | 70 | 2 | 30 | t | 20 | 0 | 10S | T | 2199 |
| IAC-24-L20 | 77 | 0 | 20 | tS | 0 | 20 | 86 | T | 2307 |
| IAC-24-L21 | 76 | 0 | 30 | 0 | 0 | 20 | 93 | T | 3031 |
| IAC-24-L24 | 85 | 0 | 30 | 0 | 0 | 10 | 90 | T | 3258 |

[a] Days from seedling emergence to heading. L.S.D. (5% level) 905
[b] For legends see Table IV.
[c] % of spikes affected by frost (sterile spikes) at maturity.

TABLE VII. ADVANCED TRIAL OF LINES SELECTED FROM IAC-24, MONTE ALEGRE DO SUL, 1990.

| Genotype | Cycle (maturity) | Lodging[a] | *Helminthosporium*[a] | Mildew[a] | Plant height (cm) | Yield[b] (kg/ha) |
|---|---|---|---|---|---|---|
| IAC-24 control | Normal | 0 | 80 | 0 | 88 | 2838 |
| IAC-24-L15 | Normal | 0 | 80 | 10 | 79 | 1791 |
| IAC-24-L16 | Normal | 0 | 30 | 0 | 92 | 2790 |
| IAC-24-L17 | Normal | 1 | 20 | 0 | 81 | 2042 |
| IAC-24-L18 | Normal | 0 | 60 | 0 | 89 | 2920 |
| IAC-24-L19 | Late | 0 | 20 | 0 | 99 | 3041 |
| IAC-24-L20 | Normal | 0 | 80 | 0 | 88 | 1441 |
| IAC-24-L21 | Normal | 0 | 60 | 0 | 94 | 2815 |
| IAC-24-L24 | Normal | 0 | 60 | 10 | 89 | 2699 |

[a] For legends see Table IV.
[b] F-test not significant.

*Advanced yield trial of genotypes selected from the cross BH 1146 x (WIN"S" AA"S")*

Tables VIII and IX present the results of Yield Trial I, which was conducted at two locations (Monte Alegre and Tatui), and included 12 mutant lines from the cross BH 1146 x (WIN"S" AA"S"). The 12 mutants all possessed a semidwarf plant type. Line L-3 produced a high yield at both locations, and L-9 was free of mildew at both locations. An additional 23 mutant lines were evaluated in Yield Trial II, at two locations (Campinas and Maracai). The results are presented in Tables X and XI. In Campinas, there were no statistical differences among the genotypes. At Maracai the lines L-16, L-21, and L-35 yielded higher than the controls. L-16 is a semidwarf and was free of rust and mildew. These genotypes were susceptible to aluminum, but they could be grown on soils low in aluminum. Line-21 possesses good agronomic traits in association with aluminum tolerance and it will be carefully observed in dry land conditions and in soils where aluminum presents a problem.

TABLE VIII. TRIAL 1 - PERFORMANCE OF CONTROLS AND 12 SELECTED LINES FROM BH1146 X (WIN'S" X AA"S"), MONTE ALEGRE DO SUL, 1990.

| Genotype | Cycle (maturity) | Lodging[a] | *Helminthosporium*[a] | Mildew[a] | Plant height (cm) | $Al^{3+}$ [a] tolerance (6 ppm) | Yield[b] (kg/ha) |
|---|---|---|---|---|---|---|---|
| BH1146 control | Early | 2 | 60 | 5 | 132 | T | 3098 |
| YAVAROS control | Normal | 1 | 60 | 5 | 80 | S | 3350 |
| L-1 | Early | 0 | 80 | 10 | 77 | S | 2859 |
| L-2 | Precocious | 0 | 60 | 0 | 82 | S | 2753 |
| L-3 | Normal | 0 | 30 | 5 | 82 | T | 3583 |
| L-4 | Normal | 0 | 60 | 10 | 82 | S | 1239 |
| L-5 | Normal | 0 | 80 | 0 | 77 | S | 1257 |
| L-6 | Early | 0 | 60 | 10 | 82 | S | 2429 |
| L-7 | Early | 0 | 60 | 0 | 89 | S | 2621 |
| L-8 | Normal | 0 | 40 | 10 | 82 | S | 1425 |
| L-9 | Normal | 0 | 40 | 0 | 80 | S | 1774 |
| L-10 | Normal | 0 | 80 | 0 | 80 | S | 1148 |
| L-11 | Normal | 0 | 80 | 10 | 91 | T | 3199 |
| L-12 | Normal | 0 | 40 | 0 | 97 | T | 2780 |

[a] For legends see Table IV.
[b] F-test not significant.

TABLE IX. TRIAL 1 - PERFORMANCE OF CONTROLS AND 12 SELECTED LINES FROM BH1146 X (WIN "S" X AAIIS"), TATUI, 1990.

| Genotype | Cycle (days) | Lodging[a] | *Helminthosporium*[a] | Leaf[a] rust | Mildew[a] | Frost[b] | Plant height (cm) | Yield (kg/ha) |
|---|---|---|---|---|---|---|---|---|
| BH-1146 Control | 70 | 2 | 30 | 10S | 20 | 0 | 130 | 2680 |
| YAVAROS Control | 85 | 0 | 10 | 0 | 10 | 0 | 72 | 1859 |
| L-1 | 70 | 0 | 30 | 0 | 20 | 100 | 85 | 956 |
| L-2 | 70 | 0 | 60 | tS | 10 | 100 | 86 | 690 |
| L-3 | 85 | 0 | 30 | 0 | 0 | 0 | 91 | 3274 |
| L-4 | 74 | 0 | 40 | 0 | 0 | 40 | 74 | 2097 |
| L-5 | 74 | 0 | 50 | 0 | 10 | 80 | 72 | 1832 |
| L-6 | 70 | 0 | 20 | 5S | 20 | 80 | 85 | 1457 |
| L-7 | 74 | 0 | 30 | 0 | 20 | 80 | 86 | 1747 |
| L-8 | 74 | 0 | 20 | 0 | 20 | 40 | 80 | 2452 |
| L-9 | 80 | 0 | 30 | tS | 0 | 60 | 91 | 1860 |
| L-10 | 83 | 0 | 40 | tS | 20 | 20 | 90 | 2475 |
| L-11 | 74 | 0 | 60 | 5S | 20 | 20 | 90 | 2428 |
| L-12 | 85 | 0 | 60 | 0 | 20 | 0 | 105 | 3911 |

[a] For legends see Table IV.
[b] For legends see Table VI.

L.S.D. (5% level) 1378

TABLE X. TRIAL 2 - PERFORMANCE OF CONTROLS AND 23 SELECTED LINES FROM BH1146 X (WIN"SUP X AA"S"), CAMPINAS, 1990.

| Genotype | Cycle (days) | Lodging[a] | *Helminthosporium*[a] | $Al^{3+}$ [a] tolerance (6 ppm) | Height (cm) | Yield[b] (kg/ha) |
|---|---|---|---|---|---|---|
| BH1146 control | 57 | 0 | 30 | T | 98 | 3579 |
| YAVAROS control | 54 | 0 | 20 | S | 80 | 3232 |
| L-13 | 65 | 3 | 20 | T | 93 | 3177 |
| L-14 | 65 | 1 | 20 | T | 95 | 2621 |
| L-15 | 65 | 0 | 10 | T | 90 | 2435 |
| L-16 | 65 | 0 | 40 | S | 75 | 2480 |
| L-17 | 55 | 1 | 30 | T | 95 | 3340 |
| L-18 | 57 | 0 | 40 | T | 85 | 3259 |
| L-19 | 65 | 0 | 10 | T | 95 | 2639 |
| L-20 | 65 | 0 | 20 | T | 95 | 2699 |
| L-21 | 76 | 0 | 20 | T | 85 | 2252 |
| L-22 | 57 | 0 | 20 | T | 100 | 3369 |
| L-23 | 52 | 0 | 20 | T | 85 | 3242 |
| L-24 | 65 | 1 | 10 | T | 105 | 2847 |
| L-25 | 65 | 0 | 20 | T | 90 | 3054 |
| L-26 | 65 | 0 | 20 | S | 90 | 2819 |
| L-27 | 65 | 0 | 30 | S | 90 | 3035 |
| L-28 | 57 | 0 | 20 | T | 90 | 3356 |
| L-29 | 65 | 0 | 10 | T | 90 | 3036 |
| L-30 | 65 | 0 | 20 | T | 90 | 2526 |
| L-31 | 63 | 0 | 30 | T | 90 | 3615 |
| L-32 | 65 | 0 | 30 | T | 80 | 3168 |
| L-33 | 65 | 0 | 20 | T | 90 | 3039 |
| L-34 | 65 | 1 | 10 | T | 85 | 3259 |
| L-35 | 63 | 0 | 30 | T | 75 | 2968 |

[a] For legends see Table IV.
[b] F-test not significant.

TABLE XI. TRIAL 2 - PERFORMANCE OF CONTROLS AND 23 SELECTED LINES FROM BH1146 X WINIISII X AA"S", MARACAI, 1990.

| Genotype | Cycle | Lodging[a] | *Helminthosporium*[a] | Rust[a] | | Mildew[a] | Height (cm) | Yield (kg/ha) |
|---|---|---|---|---|---|---|---|---|
| | | | | Leaf | Stem | | | |
| BH1146 control | Precocious | 2 | 20 | 10S | 30S | 10 | 102 | 1314 |
| YAVAROS control | Normal | 0 | 20 | 0 | 0 | 0 | 72 | 1607 |
| L-13 | Normal | 0 | 20 | 0 | 0 | 10 | 81 | 1152 |
| L-14 | Normal | 0 | 10 | 0 | 0 | 0 | 86 | 1246 |
| L-15 | Normal | 0 | 20 | 0 | 0 | 5 | 80 | 1133 |
| L-16 | Normal | 0 | 30 | 0 | 0 | 0 | 72 | 2125 |
| L-17 | Precocious | 2 | 30 | 5S | 0 | 0 | 91 | 1815 |
| L-18 | Precocious | 0 | 10 | 0 | 0 | 0 | 84 | 1551 |
| L-19 | Normal | 0 | 20 | 0 | 0 | 0 | 90 | 1086 |
| L-20 | Normal | 0 | 20 | 0 | 0 | 0 | 90 | 1046 |
| L-21 | Normal | 0 | 10 | 0 | 0 | 10 | 85 | 1959 |
| L-22 | Precocious | 0 | 20 | 0 | 30S | 10 | 100 | 1571 |
| L-23 | Precocious | 1 | 40 | 0 | tS | 0 | 80 | 1497 |

TABLE XI. (Cont.)

| Genotype | Cycle | Lodging[a] | *Helminthosporium*[a] | Rust[a] | | Mildew[a] | Height (cm) | Yield (kg/ha) |
|---|---|---|---|---|---|---|---|---|
| | | | | Leaf | Stem | | | |
| L-24 | Normal | 0 | 20 | 0 | 0 | 20 | 108 | 1599 |
| L-25 | Normal | 0 | 10 | 0 | 0 | 0 | 84 | 1136 |
| L-26 | Normal | 0 | 20 | 0 | 0 | 20 | 82 | 1140 |
| L-27 | Normal | 0 | 20 | 0 | 0 | 5 | 82 | 1004 |
| L-28 | Normal | 0 | 20 | 0 | 0 | 0 | 85 | 1567 |
| L-29 | Normal | 0 | 20 | 0 | 0 | 10 | 85 | 1441 |
| L-30 | Precocious | 0 | 30 | 0 | 0 | 0 | 81 | 1403 |
| L-31 | Normal | 0 | 10 | 5S | 10S | 10 | 82 | 1694 |
| L-32 | Normal | 0 | 10 | 5S | 10S | 10 | 82 | 1547 |
| L-33 | Normal | 0 | 40 | 5S | 10S | 5 | 85 | 1531 |
| L-34 | Normal | 0 | 10 | 0 | 10S | 10 | 85 | 1642 |
| L-35 | Precocious | 0 | 20 | 10S | 5S | 5 | 70 | 1959 |

[a] For legends see Table IV. L.S.D. (5% level) 571

Tables XII and XIII contain the results of Yield Trial III, which was conducted at Campinas and Maracai, and included 22 genotypes. In Maracai, selections L-39, L-40, L-41, L-46 and L-52 produced high grain yields compared with BH 1146, the control. The genotype L-39 was early, tolerant to aluminum, and had a lower incidence of rust. A similar genotype was L-40. At Campinas, the genotypes L-39, L-43 and L-49 showed a high yield potential and a semidwarf plant type. L-39 produced good grain yields at both locations (with and without irrigation).

Advanced Yield Trial IV, which included 23 selected lines, was conducted at Tatui and Campinas. The results are presented in Tables XIV and XV. Lines L-60, L-69, and L-71 exhibited good yield potential, a semidwarf plant type, and good disease resistance at Campinas. In Tatui the grain yields of the genotypes L-58, L-59, L-67, L-68, L-69, L-70 and L-71 were higher than those of the controls. L-50 and L-70 are of particular interest because of their level of resistance to rust, mildew and *Helminthosporium*.

Tables XVI and XVII include the results of Yield Trial V, which was conducted in Tatui and Campinas. In Campinas the soil contained aluminum. Eight lines produced yields above 2000 kg/ha. Some genotypes also had produced well in Tatui. In both locations the genotypes L-99 and L-103 showed a good association of desirable agronomic characteristics.

TABLE XII. TRIAL 3 - PERFORMANCE OF CONTROLS AND 22 SELECTED LINES FROM BH1146 X (WIN'S" X AA"S") CAMPINAS 1990.

| Genotype | Cycle (days) | Lodging[a] | *Helminthosporium*[a] | $Al^{3+}$ [a] tolerance (6 ppm) | Height (cm) | Yield (kg/ha) |
|---|---|---|---|---|---|---|
| BH1146 control | 55 | 3 | 40 | T | 93 | 2515 |
| YAVAROS control | 78 | 0 | 20 | S | 65 | 2557 |
| L-36 | 65 | 0 | 20 | T | 75 | 2405 |
| L-37 | 59 | 1 | 40 | T | 80 | 2684 |
| L-38 | 65 | 0 | 20 | S | 80 | 2446 |

TABLE XII. (Cont.)

| Genotype | Cycle (days) | Lodging[a] | *Helminthosporium*[a] | $Al^{3+}$ [a] tolerance (6 ppm) | Height (cm) | Yield (kg/ha) |
|---|---|---|---|---|---|---|
| L-39 | 65 | 0 | 20 | T | 88 | 3547 |
| L-40 | 65 | 0 | 20 | T | 50 | 2073 |
| L-41 | 59 | 0 | 20 | T | 80 | 2793 |
| L-42 | 65 | 0 | 20 | S | 70 | 1291 |
| L-43 | 59 | 1 | 20 | T | 85 | 3553 |
| L-44 | 65 | 0 | 20 | T | 93 | 2971 |
| L-45 | 65 | 0 | 20 | T | 100 | 1993 |
| L-46 | 65 | 0 | 20 | S | 75 | 2620 |
| L-47 | 65 | 0 | 20 | T | 75 | 2730 |
| L-48 | 65 | 0 | 20 | S | 75 | 2834 |
| L-49 | 65 | 0 | 20 | T | 50 | 3307 |
| L-50 | 65 | 0 | 20 | T | 85 | 2872 |
| L-51 | 72 | 0 | 20 | T | 100 | 2034 |
| L-52 | 65 | 0 | 20 | T | 95 | 2649 |
| L-53 | 65 | 0 | 20 | T | 95 | 2310 |
| L-54 | 65 | 0 | 20 | T | 95 | 2411 |
| L-55 | 72 | 0 | 20 | T | 105 | 2342 |
| L-56 | 59 | 0 | 40 | T | 85 | 2758 |
| L-57 | 59 | 0 | 20 | T | 85 | 2939 |

[a] For legends see Table IV. L.D.S (5% level) 1949

TABLE XIII. TRIAL 3 - PERFORMANCE OF CONTROLS AND 22 SELECTED LINES FROM BH1146 X (WIN'S" X AA"S") MARACAI 1990.

| Genotype | Cycle | Lodging[a] | *Helminthosporium*[a] | Rust[a] | | Mildew[a] | Height (cm) | Yield (kg/ha) |
|---|---|---|---|---|---|---|---|---|
| | | | | Leaf | Stem | | | |
| BH1146 control | Early | 2 | 20 | 10S | 20S | 0 | 95 | 1201 |
| YAVAROS control | Normal | 0 | 5 | 0 | 0 | 0 | 65 | 1851 |
| L-36 | Normal | 0 | 10 | 0 | 0 | 0 | 75 | 1344 |
| L-37 | Normal | 0 | 20 | 0 | 0 | 5 | 80 | 1290 |
| L-38 | Normal | 0 | 40 | 0 | 0 | 10 | 69 | 1675 |
| L-39 | Early | 0 | 20 | tS | 5S | 0 | 80 | 2510 |
| L-40 | Normal | 0 | 40 | 0 | 5S | 0 | 75 | 2427 |
| L-41 | Early | 0 | 20 | 20S | 10S | 0 | 80 | 2334 |
| L-42 | Normal | 0 | 20 | 30S | 10S | 20 | 68 | 1754 |
| L-43 | Normal | 0 | 40 | 0 | 20S | 0 | 82 | 1205 |
| L-44 | Normal | 0 | 20 | 0 | tS | 10 | 89 | 1503 |
| L-45 | Normal | 0 | 10 | 0 | 0 | 0 | 90 | 1197 |
| L-46 | Early | 0 | 10 | 10S | 0 | 0 | 66 | 2011 |
| L-47 | Early | 0 | 20 | 0 | 0 | 0 | 72 | 1825 |
| L-48 | Normal | 0 | 20 | 0 | 0 | 0 | 75 | 1596 |
| L-49 | Early | 0 | 20 | 0 | 0 | 10 | 66 | 1664 |
| L-50 | Normal | 0 | 20 | 0 | 0 | 0 | 80 | 1881 |
| L-51 | Normal | 0 | 40 | 0 | 0 | 0 | 85 | 1112 |
| L-52 | Normal | 0 | 30 | 0 | 0 | 10 | 87 | 1958 |
| L-53 | Normal | 0 | 20 | 0 | 0 | 0 | 90 | 1506 |
| L-54 | Normal | 0 | 20 | 0 | 0 | 0 | 80 | 992 |

TABLE XIII. (Cont.)

| Genotype | Cycle | Lodging[a] | *Helminthosporium*[a] | Rust[a] | | Mildew[a] | Height (cm) | Yield (kg/ha) |
|---|---|---|---|---|---|---|---|---|
| | | | | Leaf | Stem | | | |
| L-55 | Normal | 0 | 20 | 0 | 0 | 5 | 85 | 1878 |
| L-56 | Normal | 0 | 20 | 0 | 0 | 10 | 82 | 1652 |
| L-57 | Normal | 0 | 30 | 0 | 0 | 0 | 85 | 1322 |
| [a] For legends see Table IV. | | | | L.S.D. (5% level) | | | | 682 |

TABLE XIV. TRIAL 4 - PERFORMANCE OF CONTROLS AND 23 SELECTED LINES FROM BH1146 X (WIN'S" X AA"S") TATUI 1990.

| Genotype | Cycle (days) | Lodging[a] | *Helminthosporium*[a] | Rust[a] | | Mildew[a] | Frost[b] (%) | Plant height (cm) | Yield (kg/ha) |
|---|---|---|---|---|---|---|---|---|---|
| | | | | Leaf | Stem | | | | |
| BH1146 Control | 63 | 3 | 20 | tS | 5S | 20 | 20 | 116 | 1834 |
| YAVAROS Control | 83 | 0 | 10 | 0 | 0 | 0 | 20 | 80 | 1870 |
| L-58 | 83 | 0 | 20 | 0 | 0 | 0 | 0 | 90 | 3670 |
| L-59 | 80 | 0 | 20 | 0 | 0 | 5 | 20 | 92 | 3758 |
| L-60 | 77 | 0 | 40 | tS | 0 | 10 | 20 | 100 | 3460 |
| L-61 | 68 | 0 | 60 | 0 | 0 | 10 | 40 | 103 | 2024 |
| L-62 | 64 | 0 | 30 | tS | 0 | 0 | 20 | 90 | 1451 |
| L-63 | 70 | 0 | 30 | 0 | 0 | 5 | 20 | 87 | 2735 |
| L-64 | 77 | 0 | 40 | 5S | 0 | 5 | 20 | 91 | 2376 |
| L-65 | 80 | 1 | 40 | 0 | 0 | 20 | 0 | 105 | 2972 |
| L-66 | 83 | 0 | 10 | 5S | 0 | 30 | 0 | 88 | 3043 |
| L-67 | 64 | 0 | 40 | 0 | 0 | 10 | 20 | 97 | 3778 |
| L-68 | 77 | 0 | 30 | 0 | 0 | 5 | 0 | 95 | 4196 |
| L-69 | 80 | 0 | 20 | 0 | 0 | tS | 20 | 99 | 4431 |
| L-70 | 80 | 0 | 30 | 0 | 0 | 0 | 0 | 97 | 4327 |
| L-71 | 80 | 0 | 20 | 5S | 0 | 10 | 20 | 95 | 4283 |
| L-72 | 77 | 1 | 40 | 0 | 0 | 20 | 40 | 115 | 2980 |
| L-73 | 68 | 0 | 40 | 5S | 0 | 20 | 20 | 88 | 1712 |
| L-74 | 68 | 1 | 20 | tS | 0 | 10 | 20 | 8 | 1358 |
| L-75 | 77 | 0 | 30 | tS | 0 | 5 | 20 | 95 | 2478 |
| L-76 | 63 | 0 | 20 | 0 | 0 | 5 | 20 | 100 | 1509 |
| L-77 | 77 | 0 | 30 | tS | 0 | 5 | 20 | 96 | 2190 |
| L-78 | 68 | 0 | 20 | 5S | 0 | 5 | 10 | 95 | 2097 |
| L-79 | 80 | 0 | 10 | tS | 0 | 10 | 0 | 100 | 2697 |
| L-80 | 63 | 1 | 60 | 20S | 0 | 10 | 100 | 90 | 746 |
| [a] For legends see Table IV. | | | | | | L.S.D. (5% level) | | | 1651 |
| [b] For legends see Table VI. | | | | | | | | | |

TABLE XV. TRIAL 4 - PERFORMANCE OF CONTROLS AND 23 SELECTED LINES FROM BH1146 X (WIN'S" X AA"S") CAMPINAS 1990.

| Genotype | Cycle (days) | Lodging[a] | *Helminthosporium*[a] | Mildew[a] | Height (cm) | Yield (kg/ha) |
|---|---|---|---|---|---|---|
| BH1146 control | 57 | 3 | 10 | 0 | 95 | 2553 |
| YAVAROS control | 81 | 0 | 5 | 0 | 65 | 874 |
| L-58 | 65 | 0 | 10 | 0 | 85 | 1722 |
| L-59 | 65 | 0 | 10 | 0 | 75 | 2005 |
| L-60 | 65 | 0 | 10 | 0 | 90 | 2331 |
| L-61 | 59 | 1 | 40 | 0 | 95 | 2011 |
| L-62 | 59 | 0 | 20 | 0 | 85 | 2016 |
| L-63 | 59 | 2 | 10 | 0 | 75 | 1445 |
| L-64 | 59 | 0 | 20 | 0 | 88 | 1807 |
| L-65 | 59 | 2 | 40 | 0 | 95 | 1632 |
| L-66 | 68 | 0 | 10 | 0 | 85 | 1997 |
| L-67 | 65 | 0 | 20 | 0 | 95 | 2222 |
| L-68 | 59 | 0 | 20 | 0 | 85 | 2432 |
| L-69 | 65 | 0 | 10 | 0 | 83 | 2416 |
| L-70 | 65 | 0 | 10 | 0 | 83 | 2222 |
| L-71 | 59 | 0 | 20 | 0 | 83 | 2968 |
| L-72 | 57 | 1 | 40 | 0 | 90 | 2200 |
| L-73 | 59 | 0 | 20 | 0 | 75 | 1970 |
| L-74 | 57 | 0 | 10 | 0 | 75 | 2152 |
| L-75 | 65 | 0 | 10 | 0 | 90 | 1785 |
| L-76 | 59 | 1 | 20 | 0 | 90 | 2388 |
| L-77 | 67 | 0 | 10 | 0 | 90 | 2133 |
| L-78 | 59 | 0 | 20 | 0 | 90 | 1893 |
| L-79 | 65 | 0 | 10 | 0 | 90 | 1708 |
| L-80 | 57 | 1 | 30 | 0 | 80 | 2218 |
| [a] For legends see Table IV. | | | L.S.D. (5% level) | | | 1526 |

Yield Trial VI included 21 selected lines and was conducted at Tatui and Monte Alegre do Sul (Tables XVIII and XIX). In Tatui, the genotypes with high grain yield potential were L110, L-111, L-118, L-119, L-122, L-123 and L-124. In both locations, L-122 had a lower incidence of *Helminthosporium*. Also, in both places lines L-123 and L-124 were high yielding.

*General comments*

Numerous cultivars have been developed through mutation breeding in cereals. A recent review of *Triticum* indicates that 97 mutants were released directly to farmers as new cultivars, and 29 were included in crosses that resulted in new cultivars [10]. The mutants showed improvements over the original germplasm in such characteristics as plant height, growth cycle and resistance to various diseases. One aspect very common in mutation breeding in wheat is the so called rectification of defects of good cultivars. In our research, this approach was applied to the cultivars IAC-17, IAC-18, IAC-24 and Anahuac. Radiation increased the genetic variability in these cultivars and increased the possibility to select new, useful types. In the first yield trial, semi-dwarf types were selected as well as types with improved resistance to some diseases. Some mutant lines had higher yield potential than the original cultivars.

TABLE XVI. TRIAL 5 - PERFORMANCE OF CONTROLS AND 23 SELECTED LINES FROM BH1146 X (WIN"S" X AA"S") TATUI 1990.

| Genotype | Cycle (days) | Lodging[a] | *Helminthosporium*[a] | Rust[a] | | Mildew[a] | Frost[b] (%) | Plant height (cm) | Yield (kg/ha) |
|---|---|---|---|---|---|---|---|---|---|
| | | | | Leaf | Stem | | | | |
| BH 1146 Control | 70 | 2 | 30 | 0 | 0 | 10 | 0 | 120 | 2805 |
| YAVAROS Control | 83 | 0 | 20 | 0 | 0 | 5 | 0 | 78 | 2338 |
| L-81 | 77 | 0 | 20 | 0 | 0 | 10 | 20 | 86 | 2575 |
| L-82 | 77 | 1 | 40 | 0 | 0 | 5 | 20 | 115 | 3296 |
| L-83 | 70 | 0 | 20 | 5S | tS | 0 | 80 | 85 | 1644 |
| L-84 | 77 | 0 | 30 | 0 | 0 | 0 | 20 | 110 | 2849 |
| L-85 | 70 | 0 | 20 | 0 | 0 | 10 | 60 | 80 | 1781 |
| L-86 | 74 | 0 | 30 | 0 | 0 | 0 | 40 | 90 | 2799 |
| L-87 | 70 | 0 | 40 | 0 | 0 | 5 | 20 | 95 | 2760 |
| L-88 | 64 | 1 | 40 | 0 | 0 | 0 | 100 | 115 | 1819 |
| L-89 | 70 | 0 | 40 | 20S | 0 | 5 | 0 | 97 | 2750 |
| L-90 | 80 | 0 | 40 | 0 | 0 | 0 | 40 | 96 | 2682 |
| L-91 | 83 | 0 | 40 | 5S | 0 | 0 | 20 | 102 | 3824 |
| L-92 | 77 | 2 | 40 | 0 | 0 | 20 | 20 | 119 | 3634 |
| L-93 | 80 | 1 | 20 | 0 | 0 | 5 | 0 | 121 | 4208 |
| L-94 | 58 | 0 | 40 | 0 | 0 | 0 | 0 | 100 | 3549 |
| L-95 | 80 | 0 | 40 | 0 | 0 | 5 | 0 | 77 | 2783 |
| L-96 | 58 | 0 | 10 | tS | 0 | 0 | 0 | 100 | 3642 |
| L-97 | 80 | 0 | 40 | 0 | 0 | 10 | 20 | 85 | 2341 |
| L-98 | 86 | 0 | 10 | 0 | 0 | 0 | 0 | 96 | 3347 |
| L-99 | 80 | 0 | 30 | 0 | 0 | 0 | 0 | 96 | 4093 |
| L-100 | 80 | 0 | 20 | 0 | 0 | 0 | 0 | 100 | 3537 |
| L-101 | 80 | 0 | 20 | tS | 0 | 0 | 0 | 90 | 3449 |
| L-102 | 76 | 0 | 30 | 5S | 0 | 0 | 60 | 105 | 2100 |
| L-103 | 83 | 0 | 40 | 0 | 0 | 0 | 0 | 97 | 3865 |
| | | | | | | L.S.D. (5% level) | | | 1463 |

[a] For legends see Table IV.
[b] For legends see Table VI.

In some countries, like China, seed irradiation of hybrids obtained from wheat crosses has resulted in several cultivars [11]. The results in China indicated that irradiation promotes an increase in mutation frequency and segregation and a wider mutation spectrum [11,12]. This approach was utilized in our research, utilizing $F_4$ seeds from a cross of *T. aestivum* L. x *T. durum* L. Some lines that were higher yielding than the control were obtained and were combined with other useful traits, including semidwarf plant type and improved disease resistance. Thus far, the data indicate the potential value of the selected mutants, and further testing will be conducted to confirm their usefulness.

## 4. PRELIMINARY CONCLUSIONS

A plant breeding programme has various steps and the final one is the evaluation of the selected genotypes at different locations over years. The results presented in this paper concern the first year of evaluation of lines selected from several populations derived from seed irradiated with gamma rays. More testing is necessary before any final conclusions can be made as to the value of the mutant lines for the farmers or for use in the breeding programme. However, some general observations may be made based on the behavior of the selected mutants during the several years of research in the project.

TABLE XVII. TRIAL 5 - PERFORMANCE OF CONTROLS AND 23 SELECTED LINES FROM BH1146 X (WIN"S" X AA"S") CAMPINAS 1990.

| Genotype | Cycle (days) | Lodging[a] | *Helminthosporium*[a] | Height (cm) | $Al^{3+}$ [a] Tolerance (6 ppm) | Yield (kg/ha) |
|---|---|---|---|---|---|---|
| BH 1146 Control | 57 | 1 | 40 | 90 | T | 2352 |
| YAVAROS Control | 78 | 0 | 20 | 60 | S | 205 |
| L-81 | 55 | 2 | 40 | 70 | T | 1805 |
| L-82 | 57 | 2 | 40 | 80 | S | 1137 |
| L-83 | 65 | 1 | 20 | 70 | T | 2006 |
| L-84 | 57 | 1 | 20 | 85 | S | 1750 |
| L-85 | 57 | 2 | 40 | 70 | S | 1315 |
| L-86 | 57 | 2 | 40 | 60 | T | 1223 |
| L-87 | 63 | 1 | 40 | 70 | T | 1433 |
| L-88 | 57 | 2 | 20 | 80 | T | 879 |
| L-89 | 57 | 1 | 40 | 75 | T | 2355 |
| L-90 | 57 | 2 | 40 | 80 | T | 2316 |
| L-91 | 65 | 1 | 40 | 80 | T | 2907 |
| L-92 | 59 | 3 | 40 | 85 | S | 1490 |
| L-93 | 55 | 3 | 40 | 90 | S | 1726 |
| L-94 | 68 | 0 | 20 | 85 | S | 1811 |
| L-95 | 65 | 0 | 20 | 75 | S | 1992 |
| L-96 | 68 | 0 | 20 | 75 | T | 1688 |
| L-97 | 65 | 0 | 20 | 75 | S | 1657 |
| L-98 | 65 | 0 | 20 | 85 | T | 1289 |
| L-99 | 65 | 0 | 20 | 80 | T | 2150 |
| L-100 | 65 | 0 | 20 | 80 | T | 2163 |
| L-101 | 65 | 0 | 20 | 85 | T | 2320 |
| L-102 | 59 | 0 | 40 | 85 | T | 1679 |
| L-103 | 65 | 0 | 40 | 85 | T | 2569 |
| [a] For legends see Table IV. | | | | | L.S.D. (5% level) | 1495 |

As was expected, through the use of radiation there was an increase in genetic variability, making possible the selection of several genotypes that showed differences as compared with the original populations. In the case of the cultivars IAC-17, IAC-18 and IAC-24, genotypes were obtained that had a semidwarf plant height, an increase in resistance to diseases, and retained the tolerance to aluminum of the original cultivar. Some mutant lines produced higher yields than the controls in the 1990 yield trials but the differences were not statistically significant, and further testing will be done. If the higher yield potential of the mutants is confirmed, they should be very useful as cultivars or germplasm for the breeding programme. Because of a frost in the yield trial that included Anahuac, no data were obtained. Research with this cultivar will continue in an effort to develop a cultivar that is tolerant to aluminum and has the superior characteristics of Anahuac.

In regard to the mutant lines derived from the cross BH-1146 x (WIN"S" x AA"S"), there is strong evidence which suggests that some have high yield potential. The lines showed a combination of excellent agronomic traits and some show promise for release as new cultivars.

TABLE XVIII. TRIAL 6 - PERFORMANCE OF CONTROLS AND 21 SELECTED LINES FROM BH1146 X (WIN'S" X AA"S") TATUI 1990.

| Genotype | Cycle (days) | Lodging[a] | *Helminthosporium*[a] | Leaf[a] rust | Mildew[a] | Frost[b] (%) | Plant height (cm) | Yield (kg/ha) |
|---|---|---|---|---|---|---|---|---|
| BH 1146 Control | 63 | 4 | 20 | 5S | 0 | 0 | 115 | 2534 |
| YAVAROS Control | 87 | 0 | 20 | 0 | 10 | 20 | 84 | 1896 |
| L-104 | 80 | 1 | 60 | 0 | 10 | 40 | 76 | 2559 |
| L-105 | 77 | 0 | 40 | 0 | 5 | 40 | 80 | 2344 |
| L-106 | 80 | 0 | 40 | 0 | 5 | 20 | 75 | 2352 |
| L-107 | 77 | 0 | 20 | 0 | 10 | 20 | 80 | 2263 |
| L-108 | 77 | 0 | 60 | 0 | 0 | 0 | 80 | 2968 |
| L-109 | 74 | 0 | 40 | 0 | 0 | 60 | 85 | 2054 |
| L-110 | 82 | 0 | 40 | tS | 10 | 40 | 96 | 3298 |
| L-111 | 87 | 1 | 20 | 0 | 10 | 20 | 96 | 3806 |
| L-112 | 92 | 0 | 20 | 0 | 5 | 20 | 90 | 2942 |
| L-113 | 87 | 0 | 20 | 0 | 10 | 40 | 85 | 2937 |
| L-114 | 70 | 1 | 20 | 0 | 5 | 20 | 95 | 2479 |
| L-115 | 77 | 1 | 40 | 0 | 10 | 40 | 80 | 2625 |
| L-116 | 80 | 2 | 40 | 5S | 5 | 80 | 80 | 2245 |
| L-117 | 77 | 1 | 40 | 0 | 5 | 40 | 80 | 2653 |
| L-118 | 74 | 0 | 30 | 0 | 10 | 20 | 90 | 3122 |
| L-119 | 80 | 0 | 20 | 0 | 0 | 20 | 87 | 3181 |
| L-120 | 87 | 0 | 40 | 0 | 20 | 20 | 91 | 3035 |
| L-121 | 73 | 0 | 60 | 0 | 5 | 20 | 80 | 2069 |
| L-122 | 87 | 0 | 10 | 0 | 5 | 0 | 95 | 3110 |
| L-123 | 87 | 1 | 40 | 0 | 5 | 20 | 95 | 3298 |
| L-124 | 87 | 0 | 40 | 0 | 10 | 0 | 105 | 3199 |
| | | | | | | L.S.D. (5% level) | | 1400 |

[a] For legends see Table IV.
[b] For legends see Table VI.

TABLE XIX. TRIAL 6 - PERFORMANCE OF CONTROLS AND 21 SELECTED LINES FROM BH1146 X (WIN'S" X AA"S") MONTE ALEGRE DO SUL 1990.

| Genotype | Cycle | Lodging | *Helminthosporium* | Plant height (cm) | Yield (kg/ha) |
|---|---|---|---|---|---|
| BH 1146 Control | Early | 0 | 20 | 113 | 3568 |
| YAVAROS Control | Normal | 0 | 50 | 71 | 3732 |
| L-104 | Normal | 0 | 60 | 81 | 2393 |
| L-105 | Normal | 0 | 80 | 81 | 2253 |
| L-106 | Normal | 0 | 80 | 80 | 2116 |
| L-107 | Normal | 0 | 60 | 76 | 2620 |
| L-108 | Normal | 0 | 40 | 71 | 2602 |
| L-109 | Late | 0 | 80 | 91 | 1478 |
| L-110 | Normal | 0 | 60 | 89 | 3038 |
| L-111 | Normal | 0 | 80 | 99 | 3814 |
| L-112 | Normal | 0 | 40 | 80 | 2735 |
| L-113 | Normal | 0 | 60 | 90 | 2575 |
| L-114 | Normal | 0 | 80 | 93 | 3592 |
| L-115 | Normal | 0 | 60 | 78 | 2444 |
| L-116 | Normal | 0 | 60 | 78 | 1842 |
| L-117 | Normal | 0 | 50 | 67 | 2764 |

TABLE XIX. (Cont.)

| Genotype | Cycle | Lodging | *Helminthosporium* | Plant height (cm) | Yield (kg/ha) |
|---|---|---|---|---|---|
| L-118 | Normal | 0 | 40 | 90 | 3725 |
| L-119 | Normal | 0 | 40 | 71 | 2984 |
| L-120 | Normal | 0 | 40 | 90 | 2811 |
| L-121 | Normal | 0 | 80 | 80 | 1264 |
| L-122 | Late | 0 | 10 | 93 | 2748 |
| L-123 | Normal | 0 | 40 | 97 | 3914 |
| L-124 | Normal | 0 | 60 | 89 | 3762 |
| L.S.D (5% level) | | | | | 1679 |

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# MUTATION BREEDING OF WHEAT AND TRITICALE IN RIO GRANDE DO SUL STATE, BRAZIL

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**Abstract**

A mutation breeding programme was initiated in 1985 at IPAGRO in Rio Grande do Sul State, Brazil. Seeds from several wheat (*Triticum aestivum*) and triticale hexaploid cultivars were treated with $^{60}Co$ gamma rays and ethylmethanesulphonate (EMS) to induce mutants resistant to diseases, shorter in height, and earlier maturing. In addition, $F_1$ seeds from crosses between triticale and wheat were irradiated with gamma rays to increase translocations and recombinations. In the $M_1$ generation, the $F_1$ plants were backcrossed to triticale. In 1989, the first local replicated yield trials consisted of 1072 genotypes selected from the different populations. From these yield trials, 11 cooperative trials consisting of 245 genotypes were organized in 1990 and distributed in 3 different regions in the state. From 3 wheat trials, 14 mutant lines were selected on the basis of superior yields. Among these, some lines from RS-2 are of special interest because of reduced leaf rust. Some lines from Butuí were shorter and earlier than the original parent. Mutant lines derived from the irradiated hybrids of triticale × wheat were tested in 8 yield trials. From 4 of the trials, 28 lines were selected because of higher yields than that of the triticale control, TAS-1. In the other 4 trials the lines lacked uniformity, therefore, new selections were made. In comparing the selected genotypes of triticale with the control, several lines were superior in weight of hectoliter but no useful lines were identified for semidwarfness, earliness, or higher 1000-grain weight. Evidence for the success of the mutation breeding programme is not only the promising advanced mutant lines but also thousands of selected plants which are still being evaluated.

## 1. INTRODUCTION

The wheat and triticale crops in Rio Grande do Sul have been limited by the high frequency of diseases such as *Puccinia recondita*, *Septoria tritici*, *S. nodorum*, *Helminthosporium sativum* and *Gibberella zeae*, in addition to mosaic virus and root diseases [1,2]. Because the wheat cultivars are very tall, also lodging is a limiting factor in increasing yield [2,3,4]. In triticale, poor flour quality and low hectoliter weight are important problems that must be solved if the acreage is to increase. Another serious factor limiting the expansion of the crop in the region is that even though the triticale cultivars are generally more productive than wheat, they are less stable than the wheat cultivars [5]. The poor stability of triticale may be due to a narrow genetic base, since the origin of this cereal is so recent [6].

Because environmental conditions are very favorable for diseases and because semidwarfness appears to be associated with susceptibility, it is very difficult to find semidwarf plants with satisfactory disease resistance. In addition, due to the loss of resistance as new races of fungi appear, new cultivars must be released every year or so to replace those that have become susceptible. In triticale, which is a recent commercial crop in Brazil, the cultivars have been selections from material introduced from CIMMYT, Mexico.

Because of the problems enumerated above and others, it is important that all available breeding methods, such as mutation techniques, be utilized in conventional breeding programmes.

Micke [7] in 1973 proposed the use of induced mutations as an efficient tool for breeding disease resistant crops. Fried [8] concluded that for increasing food production in the world, inducing mutations is important in creating variability in plant breeding to improve yield, earliness, disease resistance, lodging resistance, etc. Since mutations are a source of variability in organisms and induced mutations are not essentially different from spontaneous mutations, mutation breeding is fundamental as an auxiliary to conventional plant breeding methods [9].

In the application of induced mutation methods, there are many possible ways of planning a plant breeding programme to achieve local or regional breeding objectives. Besides treating lines and cultivars with mutagens to create variability, it is possible to treat $F_1$ hybrids to promote translocations. Sears [10], the pioneer in this type of approach, used x-rays and the absence of the 5B chromosome to transfer leaf-rust resistance from *Aegilops umbellulata* to wheat. According to Knott [11], in all cases known to him, researchers used either monosomic or disomic alien addition lines of various types as the material for irradiation and the majority followed essentially the same basic procedure used by Sears. He concluded that the use of induced translocations to transfer genes for disease resistance has potential value in any species that will tolerate the addition of an alien chromosome. Since wheat carries genetic mechanisms that suppress pairing of homeologous chromosomes, the transfer of genes by crossing over is difficult.

It is known that meiotic irregularities increase in irradiated materials. In triticale, x-ray treatment increased the frequency of bridges and fragments in comparison with the control [12]. These meiotic irregularities can offer the possibility of translocations even without the use of the absence of the 5B chromosome. It is not only homeologous pairing that is useful. Depending on the objectives, homeologous pairing combined with irradiation can present an increase in recombinations and a transfer of genes.

Based on these possibilities, an agreement between IPAGRO and CENA was initiated in 1985, with the main objective of developing cultivars with resistance to diseases, semidwarfism, and earliness in wheat and triticale, and increasing recombinations in triticale × wheat crosses. The ultimate aim has been to create variability from which to develop cultivars at least 5% more productive than the controls. Seeds of local wheat and triticale cultivars and seeds from crosses have been treated with gamma rays of $^{60}Co$ and ethyl methane sulphonate (EMS). The objectives were to create variability through gene mutation and to transfer characters from wheat to triticale by translocations or recombinations, with the aid of gamma ray treatments.

Specifically, this project has concentrated on getting resistance to leaf-rust and *Helminthosporium* on leaves, earliness, and semidwarfness in wheat cultivars (RS-1, RS-2, Butuí, Jacuí, and CEP 17), in addition to resistance to scab. For crosses between triticale and wheat (TSA-1 x Jacuí), the specific objectives have been the improvement in flour quality, weight of hectoliter of grain, and scab resistance.

## 2. MATERIALS AND METHODS

This project is being conducted at the IPAGRO Experimental Station located at Julio de Castilho in Rio Grande do Sul, Brazil. The physiographic region is identified as the "Planalto Riograndense" and is characterized by a subtropical climate, with hot and rainy springs.

Gamma rays from $^{60}Co$ and EMS are being used to induce mutations in wheat and triticale. To determine the sensitivity of wheat and triticale to irradiation, seeds of the local cultivars were subjected to irradiation dosages ranging from 10 to 55 krad (100-500 Gy) in increments of 5 or 10

krad at a rate of 172 krad/hour. The sensitivity determinations were conducted at CENA. The moisture content of the treated seed was 12%, and each treatment was replicated 3 times. Based on seedling height, survival, and plant development, two dosages usually were selected and 5 kg of seed/dose were treated (Table I). For the seed from the triticale × wheat crosses, 4000 to 20 000 seed were treated. The seed was derived from approximately 5000 spikes that had been emasculated and pollinated.

TABLE I. GENOTYPES OF WHEAT, TRITICALE AND TRITICALE × WHEAT HYBRIDS, MUTAGENIC TREATMENTS, AMOUNTS OF SEEDS AND YEARS.

| Years | Genotypes | Gamma rays (krad) and EMS conc. | Amount of seeds |
|---|---|---|---|
| 1985 | RS 1 Fênix | 25.0 and 27.5 | 10 kg |
| | RS 2 Palmeira | 35.0 and 36.5 | 10 kg |
| | TSA-1 (Tcl) | 20.0 and 25.0 | 10 kg |
| 1986 | Butuí | 25.0 and 5 | 10 kg |
| | RS 4 | 35.0 and 42. | 10 kg |
| | Jacuí | 27.5 and 32.5 | 10 kg |
| | BR 1 (Tcl) | 35.0 and 40.0 | 10 kg |
| | TSA 1/Jacuí | 30.0 | 4000 seeds |
| | TSA 1/S-8017 | 30.0 | 4000 seeds |
| 1987 | CEP 17.0 | 40.0 | 10 kg |
| | CEP 18.0 (Tcl) | 30.0 | 10 kg |
| | CEP 17 | 0.1 M, EMS, 2hr | 8000 seeds |
| | CEP 18 (Tcl) | 0.1 M, EMS, 2hr | 8000 seeds |
| 1988 | TSA 1/Jacuí | 30.0 | 1000 seeds |
| 1989 | TSA 1/Jacuí | 30.0 | 20 000 seeds |
| 1986/87 (summer) | RS 1 Fênix | 25.0 and 27.5 | 4 kg |
| 1969/90 (summer) | BR 34 | 40.0 and 44.0 | 7 kg |

The EMS treatments were made at the IPAGRO Experimental Station, and consisted of treating pre-soaked seed (8000 seed/treatment) with 0.1 M EMS at 35°C for 2 hours [13].

The $M_1$ populations were usually grown in the field and received fungicides, manual weed control, and irrigation as needed. No selection was practiced in the $M_1$ generation. The $M_1$ populations were bulk harvested except for the EMS-treated populations, in which all spikes were harvested individually. The controls were grown along with the respective treated populations in the field and greenhouse and received the same cultural inputs.

Selection for semidwarfness and earliness began in the $M_2$ generation. Selection began in the $M_3$ generation for disease resistance (primarily leaf rust and scab), 1000 grain weight, grain yield, seed volume, hectoliter weight, flour quality and grain features. Because of the difficulty of working

with thousands of lines and variations in the quantity of seed available, the weight of 140 ml of seed was used to represent the hectoliter weight.

Plant height was measured at maturity, and earliness was based on the difference in number of days to flowering between plants or lines and their respective controls. The evaluation of flour quality was based on the Pelshenke test: the time of resistance of flour under fermentation in warm water (30°C). Disease ratings were based on the international scales used by CIMMYT and in common use by research institutions in Brazil.

Selected individual plants became lines in subsequent populations. The selected lines were included in yield trials using a randomized block design with 4 replications and individual plots consisting of 2 rows x 2 m, with a 0.2 m spacing between rows. In the final experiments the plot size was increased to 5 rows x 5 m. In all cases, the seeding rate has been 300 seed/$m^2$. The experiments were conducted in 4 regions: Julio de Castilhos, Santo Agusto, Sao Borja, and Veranopolis. In the $M_2$ generations derived from wheat cultivars, approximately 6000 plants were selected from 1986 to 1989, resulting in 418 lines tested in local trials in the field in 1989, and 61 lines in 1990.

In the triticale x wheat crosses, three $M_1$ $F_1$ generations with backcrosses provided 8000 selected plants up to 1990, resulting in 654 lines tested in trials in 1989 and 184 lines in cooperative trials in three different environments in 1990. It is on the performance in these cooperative trials that decisions are made to approve or disapprove the release of an experimental line as a new cultivar.

In 1991, seed increases of selected mutant lines from the cooperative yield trials were conducted. These superior mutant lines offer two possibilities, (1) direct use as new cultivars or (2) parents in crosses in the breeding programme.

In 1991 there were thousands of selected plants and lines from cultivars and from triticale × wheat crosses. It was from these populations that the next cycle of yield trials was to be organized.

## 3. RESULTS AND DISCUSSION

The results that are considered most useful are summarized in Tables II to VII. Pelshenke test data are not presented because they are inconsistent and must be combined with other laboratory tests like microsedimentation, alveogram, etc. Cytological analyses and electrophoretic studies are planned for the near future.

*Determination of gamma ray sensitivity*

Radiation dosages were based on sensitivity trials conducted at CENA. The variation in the dosages presented in Table I indicates that there were large differences in the sensitivity of the cultivars and the crosses to gamma rays. For wheat, the results of the sensitivity trials conducted at CENA were similar to experiences in the field at Julio de Castilhos; however, triticale cultivars exhibited more sensitivity in the field than at CENA, and most of the material did not survive, with no useful mutants recovered. It may be necessary to make adjustments in the method of measuring triticale sensitivity.

*The $M_1$ and $M_2$ generations*

Table I presents the irradiation dosages, EMS treatments, and the various quantities of seed treated. Each 10 kg contained about 250 000 seeds. For the triticale x wheat crosses it was necessary to emasculate and pollinate thousands of spikes because in these crosses each spike produced only about 3 or 4 seeds. Except for the EMS treated populations, the $M_1$ populations were bulk harvested, which provided a large quantity of seed for the $M_2$ populations. The $M_2$ populations ranged from 60 to 5000 $m^2$ in area, and many semidwarf and early mutants were selected (Table II).

TABLE II. SELECTIONS REALIZED IN $M_2$, $M_3$ AND $M_4$ GENERATIONS WHICH ORIGINATED THE LINES TESTED IN THE YIELD TRIALS IN 1989 AND 1990.

| Genotypes | Dwarfness | | Earliness | | Various* | |
|---|---|---|---|---|---|---|
| | 1987 | 1988 | 1987 | 1988 | 1987 | 1988 |
| | No. of plants | | No. of plants | | No. of plants | |
| Butuí 25.0 krad | 194 | 170 | 69 | 58 | 90 | 48 |
| Butuí 32.5 krad | 30 | 119 | 7 | 35 | 28 | 59 |
| RS 4 35.0 krad | 248 | 21 | 121 | 226 | 52 | 157 |
| RS 4 42.5 krad | 243 | 52 | 11 | 63 | 77 | - |
| Jacuí 27.5 krad | 103 | 47 | 233 | 55 | - | 49 |
| Jacuí 32.5 krad | 31 | - | 49 | 218 | 20 | - |
| BR 1 40.0 krad | - | - | - | - | - | - |
| BR 1 35.0 krad | 154 | - | 32 | - | 33 | - |
| RS 1 25.0 + 27.5 krad | 66 | - | 17 | 46 | 6 | - |
| RS 2 35.0 krad | - | - | 55 | - | 12 | - |
| RS 2 38.5 krad | - | - | 48 | - | 23 | - |
| $M_2$ crosses 30.0 krad | - | - | - | - | 150 | - |
| $M_2$ backcross 30.0 krad | - | - | - | - | 2475 | - |
| CEP 17 30.0 krad | - | 63 | - | 24 | - | 227 |
| CEP 18 30.0 krad | - | 337 | - | 271 | - | - |
| CEP 17 EMS | - | 50 | - | 100 | - | - |
| CEP IS EMS | - | B9 | - | - | - | - |
| | No. of lines | | No. of lines | | No. of lines | |
| RS 1 25.0 + 27.5 krad | 27 | - | - | 8 | - | - |
| Jacuí 27.5 krad | - | 7 | - | 89 | - | - |
| Jacuí 32.5 krad | - | 2 | - | 20 | - | - |
| RS 4 35.0 krad | - | 6 | - | 76 | - | - |
| RS 4 42.5 krad | - | 11 | - | 17 | - | 2 |
| Butuí 25.0 krad | - | - | - | 57 | | 7 |
| Butuí 32.5 krad | - | 2 | - | 27 | | - |
| BR-1 35.0 krad | - | 5 | - | 5 | - | 17 |
| RS-2 35.0 krad | - | 1 | - | 9 | - | - |
| RS 2 38.5 krad | - | - | - | 18 | - | 5 |
| $M_3$ crosses and backcrosses | - | - | - | - | - | 654 |

* Includes disease resistance, agronomic characteristics etc.

*The $M_3$ and subsequent generations*

One spike per selected plant was harvested in the $M_2$ generation. In the case of disease resistance, all of the $M_3$ seed was harvested, and the $M_3$ populations sometimes contained hundreds of thousands of plants, as in RS-1, RS-4, and Butuí.

*Results from selection in the $M_2$, $M_3$, and $M_4$ generations*

All plant selections were made under field conditions. Seeds of each selected plant were visually evaluated in the laboratory and those that were retained became lines in the next generation. Table II presents data on the number of putative mutant plants selected from the various populations in 1987 and 1988 and the number of lines that were derived from these plants. Of 1069 plants identified as semidwarfs in 1987, only 34 proved to be semidwarfs in the subsequent generation in 1988. The confirmation rate for earliness was considerably higher, with 326 lines confirmed as early in 1988 out of 647 plants identified as early in 1987. The confirmation rate for disease resistance, agronomic traits, etc. was very low, only 31 lines from 341 plants.

No mutant lines from the wheat cultivar CEP 17 or the triticale cultivar CEP 18 were included in advanced trials. Lines from these populations, which received gamma ray and EMS treatments, were still only in preliminary trials.

The triticale cultivar BR-1, in spite of having a relatively large number of plants classified as semidwarfs or early maturing, provided only 10 lines in 1988. From the 40 krad treated population no useful mutants were identified. The triticale cultivar TSA-1 is absent from Table II because there were no visible differences between the treated populations and the control.

*Results from selection in triticale × wheat hybrids*

The first triticale × wheat crosses were made in 1986 (Table I). The TSA-1 × Jacuí cross produced more seeds than that of TSA-1 x S-8017. Even after backcrossing to triticale, the TSA-1 × S-8017 was degenerating, with many fertility problems due to meiotic irregularities that were detectable in the field and in the laboratory. Routine investigations in the laboratory of the Federal University of Santa Maria in Rio Grande do Sul State clearly demonstrated that these irregularities were extremely high in TSA-1 × S-8017. This was also evident in the field, since few lines were selected from the cross involving S-8017 and there are no lines from this material in advanced trials.

Table II shows the far greater number of selections obtained from the triticale × wheat backcrosses compared with the single crosses, apparently because of fertility problems in the latter populations. From 2625 plants selected for disease resistance and improved agronomic traits in the backcross populations in 1987, 654 lines were selected in 1988 on the basis of grain yield, 1000 grain weight, seed volume, weight of the hectoliter, and disease resistance.

For these populations, the controls were the parents and the crosses that received no irradiation treatment. Field observations seemed to demonstrate that fertility in the treated populations was not lower than in the respective cross controls.

Essentially none of the semi-dwarf selections in these populations was useful, apparently as a result of deleterious factors due to the nature of the cross. Also, no early maturing plants were identified.

*Cooperative yield trials of genotypes selected from Jacuí, RS-4, Butuí, and RS-2.*

Tables III, IV, and V present results of yield trials conducted at Julio de Castilhos, Sao Borja, and San Agusto, each situated in a different physiographic region of the state.

TABLE III. RESULTS OF 1990 TRIALS IN 3 REGIONS (1=JÚLIO DE CASTILHOS, 2=SANTO AUGUSTO AND 3=SÃO BORJA) OF RIO GRANDE DO SUL STATE WITH LINES SELECTED FROM JACUÍ, RS-4 AND BUTUÍ.

| Genotypes | Yield (kg/ha)-means | | | Region 1 | | | | | | Region 3 |
|---|---|---|---|---|---|---|---|---|---|---|
| | Region 1 | Region 2 | Region 3 | Weight of (g) | | Date of flowering | Leaf rust | Leaf S/H[b] | Glume S/H[c] | Plant height (cm) |
| | | | | 1000 seeds | 140 ml of seeds | | | | | |
| Jacuí control | 1152 | 2270 | 2288 | 30.3 | 84.5 | 30/09 | 0 | 3 | 4+ | 100 |
| Jacuí 9044 | 1319 | 2520[a] | 2669 | 30.0 | 86.5 | 23/09 | 0 | 2 | 4 | 95 |
| Jacuí 9046 | 1704[a] | 2583[a] | 3288[a] | 32.5 | 88.5[a] | 17/09 | TR | 3 | 3 | 100 |
| Jacuí 9050 | 1250 | 2291 | 2538 | 33.5 | 88.8[a] | 18/09 | 0 | 3 | 4+ | 105 |
| RS-4 control | 1894 | 2833 | 3387 | 29.6 | 90.5 | 18/09 | 15MS | 3+ | 4 | 100 |
| RS-4 9054 | 2285 | 2979 | 3681 | 29.3 | 91.5 | 22/09 | TMS | 2 | 3+ | 90 |
| Butuí control | 1523 | 2208 | 2638 | 31.2 | 88.8 | 22/09 | 30S | 2+ | 4 | 100 |
| Butuí 9059 | 1419 | 1979 | 2738 | 28.4 | 85.5 | 26/09 | 0 | 3 | 4 | 95 |
| Butuí 9061 | 1417 | 1979 | 2825 | 31.5 | 85.2 | 20/09 | 5S | 3 | 4+ | 95 |
| Butuí 9062 | 1748 | 2250 | 3194 | 35.3 | 90.0 | 20/09 | 5S | 3 | 4+ | 95 |

[a] Denotes significance at the 5% level, Duncan test, compared with respective control.
[b] Complex of *Septoria* and *Helminthosporium* on the leaves.
[c] Complex of *Septoria* and *Helminthosporium* on the glumes.

Table III includes several promising lines. Jacuí 9044 yielded more than the control at one location. Jacuí 9046 was superior to the control in all locations in respect to yield and hectoliter weight, and was 13 days earlier. Three selections from Butuí, particularly Butuí 9059, exhibited good resistance to leaf-rust.

Table IV includes data from a yield trial that included 9 mutant lines derived from Butuí and was conducted in 3 regions in 1990. One selection, Butuí 9077, yielded more than the control at two of the three test sites and performed better than the control in respect to weight of the hectoliter, leaf-rust resistance, tolerance to *Helminthosporium* and *Septoria* on the leaves and glumes, and was 15 days earlier. With only one exception, all of the mutant lines included in Table IV were superior to the control for leaf-rust resistance.

Table V presents the results from the cooperative yield trial number 6, which was grown in 2 regions and included mutant lines from Butuí and RS-2. One of the lines, Butuí 9084, is of particular interest because it combines semidwarfism and satisfactory leaf-rust resistance and it was the most tolerant line to *Helminthosporium* and *Septoria* complex on the leaves and glumes. Several lines from Butuí were better than the control in yield, seed weight, and disease resistance. In the case of RS-2, some lines, particularly RS-2/9098 and RS-2/90100, had confirmed rust-resistance for the past 3 years and are two of the most important lines obtained in the project, because RS-2 is one of the most susceptible cultivars in the state.

TABLE IV. RESULTS OF 1990 TRIALS IN 3 REGIONS (1=JULIO DE CASTILHOS, 2=SANTO AUGUSTO AND 3=SAO BORJA) OF RIO GRANDE DO SUL STATE WITH LINES SELECTED FROM Butuí.

| Genotypes | Yield (kg/ha)-means | | | Region 1 | | | | | | Region 3 |
|---|---|---|---|---|---|---|---|---|---|---|
| | Region 1 | Region 2 | Region 3 | Weight of (g) 1000 seeds | 140 ml of seeds | Date of flowering | Leaf rust | Leaf S/H[b] | Glume S/H[c] | Plant height (cm) |
| Butuí control | 1448 | 2146 | 2938 | 29.2 | 88.5 | 25/09 | 40S | 4 | 4+ | 100 |
| Butuí 9065 | 1305 | 1979 | 2981 | 36.8[a] | 87.0 | 26/09 | 5MS | 3 | 4 | 90 |
| Butuí 9066 | 1192 | 2396[a] | 3338[a] | 30.2 | 82.0 | 19/09 | 5MS | 3 | 4+ | 85 |
| Butuí 9067 | 1371 | 1708 | 2844 | 37.0[a] | 85.0 | 20/09 | 5MS | 2 | 4+ | 85 |
| Butuí 9068 | 1469 | 2229 | 3481[a] | 33.5 | 87.0 | 26/09 | 5MS | 2 | 4 | 90 |
| Butuí 9070 | 1794[a] | 2062 | 2950 | 37.5[a] | 86.2 | 24/09 | 10S | 2+ | 3+ | 90 |
| Butuí 9074 | 2112[a] | 1625 | 2550 | 29.8 | 89.2 | 20/09 | 5MS | 2+ | 3 | 100 |
| Butuí 9076 | 2052[a] | 1625 | 3637[a] | 29.5 | 92.8[a] | 21/09 | 10S | 3 | 4 | 95 |
| Butuí 9077 | 2277[a] | 1854 | 3437[a] | 31.8 | 96.0[a] | 12/09 | 10MS | 3 | 2+ | 95 |
| Butuí 9083 | 1931[a] | 1875 | 3381[a] | 32.8 | 90.2 | 10/09 | 40S | 3 | 4 | 85 |

[a] Denotes significance at the 5% level, Duncan test, compared with respective control.
[b] Complex of *Septoria* and *Helminthosporium* on the leaves.
[c] Complex of *Septoria* and *Helminthosporium* on the glumes.

TABLE V. RESULTS OF 1990 TRIALS IN 2 REGIONS (1 = JULIO DE CASTILHOS AND 3= SAO BORJA) OF RIO GRANDE DO SUL STATE, WITH LINES SELECTED FROM Butuí AND RS-2.

| Genotypes | Region 1 | Region 3 | Region 1 | | | | | | Region 3 |
|---|---|---|---|---|---|---|---|---|---|
| | | | Weight of (g) 1000 seeds of | 140 ml seeds | Date of flowering | Leaf rust | Leaf S/H[b] | Glume S/H[c] | Plant height (cm) |
| Butuí control | 1585 | 2988 | 28.8 | 95.2 | 26/09 | 40S | 4 | 4+ | 95 |
| Butuí 9084 | 1754 | 2850 | 33.2[a] | 90.8 | 20/09 | 10MS | 2 | 3+ | 80 |
| Butuí 9088 | 1762 | 2831 | 35.0[a] | 92.5 | 19/09 | 20S | 2 | 4+ | 95 |
| Butuí 9089 | 2108[a] | 2325 | 28.0 | 96.2 | 12/09 | 20S | 2+ | 4+ | 90 |
| Butuí 9091 | 2375[a] | 3494 | 31.2 | 99.8[a] | 14/09 | 10MS | 2+ | 3+ | 95 |
| Butuí 9092 | 1769 | 2694 | 33.2[a] | 90.5 | 10/09 | 20S | 24 | 4+ | 85 |
| Butuí 9094 | 2175[a] | 2819 | 35.3[a] | 88.2 | 13/09 | 5MS | 24 | 3 | 90 |
| RS-2 control | 1752 | 3702 | 26.5 | 92.5 | 13/09 | 50S | 4 | 4+ | 90 |
| RS-2 9096 | 2179 | 2869 | 30.0 | 90.5 | 13/09 | 40S | 4 | 4 | 85 |
| RS-2 9097 | 2000 | 2988 | 31.5[a] | 99.0[a] | 09/09 | 40S | 4 | 4 | 85 |
| RS-2 9098 | 2462[a] | 3612 | 30.5[a] | 101.0[a] | 18/09 | 10MS | 3 | 4 | 95 |
| RS-2 9099 | 1884 | 3525 | 29.5 | 97.8[a] | 18/09 | 10MS | 3 | 4+ | 95 |
| RS-2 90100 | 2304[a] | 3194 | 29.0 | 100.0[a] | 18/09 | 5MS | 2 | 4 | 95 |
| RS-2 90102 | 2019 | 3339 | 27.0 | 93.8 | 17/09 | 50S | 4 | 4+ | 95 |

[a] Denotes significance at the 5% level, Duncan test, compared with respective control.
[b] Complex of *Septoria* and *Helminthosporium* on the leaves.
[c] Complex of *Septoria* and *Helminthosporium* on the glumes.

Table VI contains a summary of some of the selected mutant lines that have been included in the cooperative yield trials. In respect to grain yield, leaf-rust resistance and earliness , several lines had confirmed superior performance over a 3-year period, 1989, 1990, and 1991. In spite of the large number of selections for semidwarfism, the trait was confirmed in only one line, Butuí 9084. It is considered one of the most important mutant lines selected in the project.

TABLE VI. PERFORMANCE OVER 3 YEARS OF SOME SELECTED LINES IN COOPERATIVE YIELD TRIALS.

| Genotypes | Years and traits | | | |
|---|---|---|---|---|
| | 1988 | | 1989 | 1990 |
| | selected for | kg/ha[a] | distinct for | distinct for |
| Jacuí control | | 2875 | | |
| Jacuí 9044 | earliness | - | earliness | yield, earliness |
| Jacuí 9046 | earliness | 3258 | earliness | yield, earliness |
| Jacuí 9050 | earliness | 3312 | earliness, hectoliter weight | earliness, hectoliter weight |
| RS 4 control | | 3330 | | |
| RS 4 9054 | leaf-rust resistance | 4171 | leaf-rust resistance | leaf-rust resistance |
| Butuí control | | 3125 | | |
| Butuí 9059 | leaf-rust resistance | - | leaf-rust resistance | leaf-rust resistance |
| Butuí 9061 | leaf-rust resistance | 3908 | hectoliter seed weight | leaf-rust resistance |
| Butuí 9062 | good aspect | - | hectoliter weight | leaf-rust resistance |
| Butuí 9065 | good aspect | 4096 | seeds weight | seed weight |
| Butuí 9066 | earliness | 4371 | | yield, leaf-rust resistance |
| Butuí 9067 | leaf-rust resistance | 3975 | | leaf-rust resistance |
| Butuí 9068 | leaf-rust resistance | 4033 | leaf-rust resistance | yield, leaf-rust resistance |
| Butuí 9070 | good aspect | 3733 | | yield, hectoliter weight |
| Butuí 9074 | leaf-rust resistance | - | leaf-rust resistance | leaf-rust resistance |
| Butuí 9076 | good aspect | 4258 | | yield, hectoliter weight |
| Butuí 9077 | earliness | 4729 | earliness | yield, earliness |
| Butuí 9083 | earliness | 4488 | earliness | yield, earliness |
| Butuí 9084 | plant height | 4500 | | plant height |
| Butuí 9088 | leaf-rust resistance | 4583 | leaf-rust resistance | earliness, seed weight |
| Butuí 9089 | earliness | 3692 | hectoliter weight | earliness |
| Butuí 9091 | earliness | 5120 | hectoliter weight, earliness | yield, earliness |
| Butuí 9092 | earliness | 3980 | hectoliter weight, earliness | earliness, seed |
| Butuí 9094 | good aspect, earliness | 4900 | hectoliter weight, seed weight | earliness, leaf-rust resistance |
| RS 2 control | | 3679 | | |
| RS 2 9096 | good aspect | 3971 | | |
| RS 2 9097 | earliness, good aspect | - | earliness, good aspect | seed weight |
| RS 2 9098 | leaf-rust resistance | 4550 | leaf-rust resistance | leaf-rust resistance, yield |
| RS 2 9099 | leaf-rust resistance | 4500 | leaf-rust resistance | leaf-rust resistance, hectoliter weight |
| RS 2 90100 | leaf-rust resistance | 4829 | leaf-rust resistance | leaf-rust resistance, hectoliter weight |
| RS 2 90102 | good aspect | - | | |

[a] Means from local yield trials (Júlio de Castilhos) with 4 replications. All the presented means are statistically superior to their respective control.

*Cooperative yield trials of genotypes selected from crosses between triticale and wheat*

Eight yield trials were conducted to test the performance of mutant lines selected from the crosses of triticale × wheat. However, because the majority of the lines in some tests were not sufficiently uniform, Table VII includes the results of only 4 trials in 3 environments (2 locations and a second sowing time in Julio de Castilhos). Several lines were superior to the control for grain yield and weight of the hectoliter but none had a greater 1000 grain weight. The lines TSA-1 90128, 90154, 90182, 90184 90187, and 90190 are probably some of the most useful ones developed in the project. The lines TSA-1 90158, 90160, 90163, 90165, and 90166 are of interest primarily because of the relatively high hectoliter weight.

## 4. GENERAL CONSIDERATIONS

Through mutation breeding in *Triticum*, several cultivars have been directly released to producers or included in cross breeding programmes from which new cultivars were developed [14]. These mutants have been useful for traits like disease resistance, plant height, earliness, and adaptability.

This project has demonstrated that induced mutations created sufficient variability to achieve the proposed goals. Some mutant traits were recovered more easily than others, e.g., earliness compared with semidwarfness.

The majority of the lines in 1988 originated from selections for earliness in 1987. Because of strong environmental influences, none of the lines selected for semidwarfism, with one exception, proved to be such. The advanced lines are being tested in additional yield trials and may be able to replace the original cultivars, or be used as parents in the conventional breeding programme.

No triticale mutant lines were included in yield trials because the selected plants and lines had many defects. In the populations from the triticale × wheat crosses no useful semidwarfs were identified. However, semidwarfs were not a main objective with the cross populations because triticale cultivars have strong culms and are relatively resistant to lodging under regional conditions. No early maturing mutants were obtained from the cross populations.

Recently, others have been continuing the work initiated by Sears, transferring genes by induced translocations. Using, for example, polyhaploids for the development of reciprocal translocation homozygotes [15], or using the *ph 1b* mutation as the pairing induction system [16]. In a different approach, in China [17] several cultivars have been developed by irradiating seeds of wheat hybrids, using the homeologous pairing to increase recombinations.

New research findings on the effects of radiation on mitosis and meiosis give similar results. One report on this subject in wheat indicated that abnormalities, e.g. laggards, bridges, chromatin stickiness etc. in meiosis and mitosis increase with rising radiation dosages [18]. These irregularities may offer the possibility of translocations in some selected lines, even without homeologous pairing.

TABLE VII. RESULTS OF 4 TRIALS IN 3 ENVIRONMENTS (I = JÚLIO DE CASTILHOS, FIRST SOWING TIME; 2 = SANTO AUGUSTO; 3 = JÚLIO DE CASTILHOS, 2ND SOWING TIME), INCLUDING SELECTED LINES FROM THE CROSS TRITICALE X WHEAT, ORIGINALLY IRRADIATED WITH 30 KRAD OF GAMMA RAYS.

| | Yield (kg/ha)-means | | | Envir. 1 weight of (g) | |
|---|---|---|---|---|---|
| | Envir.1 | Envir.2 | Envir.3 | 1000 seeds | 140 ml of seeds |
| Trial VII | | | | | |
| TSA-1 control | 2308 | 2385 | 1464 | 33.7 | 72.2 |
| TSA-1 90110 | 2433 | 2864[a] | 1771[a] | 33.8 | 77.0[a] |
| TSA-1 90117 | 2417 | 2885[a] | 1735[a] | 33.0 | 77.5[a] |
| TSA-1 90122 | 2775[a] | 2573 | 1396 | 33.4 | 79.3[a] |
| TSA-1 90124 | 2776[a] | 2521 | 1448 | 34.7 | 80.0[a] |
| TSA-1 90125 | 2817[a] | 2656 | 1427 | 34.3 | 78.0[a] |
| Trial VIII | | | | | |
| TSA-1 control | 2017 | 2396 | 1417 | 30.8 | 72.0 |
| TSA-1 90128 | 2750[a] | 2750[a] | 1758[a] | 30.7 | 77.0[a] |
| TSA-1 90136 | 2333[a] | 2740[a] | 1544 | 31.5 | 74.0 |
| TSA-1 90137 | 2667[a] | 2656 | 1619[a] | 31.0 | 71.7 |
| TSA-1 90139 | 2108 | 2583 | 1944[a] | 32.8 | 76.7[a] |
| TSA-1 90145 | 2800[a] | 2396 | 1677[a] | 30.8 | 74.7 |
| TSA-1 90146 | 2608[a] | 2573 | 1798[a] | 30.0 | 76.7[a] |
| TSA-1 90147 | 2892[a] | 2458 | 1958[a] | 31.0 | 73.0 |
| Trial IX | | | | | |
| TSA-1 control | 2443 | 2551 | 1348 | 32.6 | 73.8 |
| TSA-1 90150 | 2235 | 2750[a] | 1690[a] | 32.2 | 79.2[a] |
| TSA-1 90151 | 2783[a] | 2698 | 1408 | 34.5 | 79.5[a] |
| TSA-1 90152 | 2996[a] | 2719[a] | 1290 | 33.5 | 76.5 |
| TSA-1 90154 | 3173[a] | 2917[a] | 1677[a] | 36.0 | 81.5[a] |
| TSA-1 90155 | 2781[a] | 2719[a] | 1396 | 32.5 | 74.0 |
| TSA-1 90156 | 2785[a] | 2760[a] | 1533 | 36.0 | 76.5 |
| TSA-1 90158 | 2110 | 2031 | 1596[a] | 35.5 | 101.5[a] |
| TSA-1 90160 | 2219 | 1958 | 2202[a] | 32.5 | 101.2[a] |
| TSA-1 90163 | 2721 | 2364 | 1979[a] | 36.5 | 92.0[a] |
| TSA-1 90165 | 2517 | 1938 | 2033[a] | 32.0 | 103.0[a] |
| TSA-1 90166 | 2131 | 1875 | 1481 | 30.0 | 95.8[a] |
| Trial X | | | | | |
| TSA-1 control | 1888 | 2208 | 1150 | 32.2 | 67.2 |
| TSA-1 90181 | 2096 | 2854[a] | 1625[a] | 33.5 | 74.8[a] |
| TSA-1 90182 | 2779[a] | 2885[a] | 1554[a] | 32.5 | 74.5[a] |
| TSA-1 90183 | 2088 | 2864[a] | 1981[a] | 33.5 | 72.7[a] |
| TSA-1 90184 | 2883[a] | 2906[a] | 1704[a] | 32.5 | 76.5[a] |
| TSA-1 90187 | 2596[a] | 2585[a] | 1738[a] | 32.5 | 73.8[a] |
| TSA-1 90190 | 2762[a] | 2667[a] | 1662[a] | 32.5 | 76.0[a] |

[a] Denotes significance at the 5% level, Duncan test, compared with the respective control.

## 5. CONCLUSIONS

The results obtained in this work permit some important conclusions:

1. The main objective of creating variability was achieved. It was clearly observable in the field and proven by the experimental results obtained.

2. Mutant lines with leaf-rust resistance, earliness, semi-dwarfness, and excellent yield performance relative to the respective controls, indicate that through induced mutations it is possible to select mutants for direct release as varieties for the farmers.

3. The method has great potential because it can produce useful alleles in a short time.

4. In spite of the potential for direct release of mutants as new cultivars, induced mutations should be considered as complementary to conventional breeding programmes, because most mutants serve as sources of desirable genes. Segregating populations derived from crosses form the foundation of conventional breeding programmes and will continue in importance.

5. Earliness appears to be very beneficial, since several lines with this trait were superior to the respective controls for other characteristics like yield, 1000 grain weight, hectoliter weight and leaf-rust resistance. It may be that the early mutant lines were more productive because they escaped diseases that reduced the yields of later maturing genotypes.

6. The great variability in the populations from the irradiated $F_1$ seeds from triticale $\times$ wheat crosses provides the programme with many possibilities for selections.

7. From the irradiated triticale $\times$ wheat crosses it was possible to find improved lines for hectoliter weight associated with improvement in yield.

8. The occurrence of several triticale lines with relatively high hectoliter weight is a strong evidence of the transference of this trait from wheat into triticale.

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# MUTANT SELECTIONS FROM WHEAT CULTIVAR IAPAR 3-ARACATU WITH REDUCED HEIGHT

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**Abstract**

The development of high yielding, widely adapted, and stable wheat cultivars is the main goal of the breeding project at IAPAR. More recently, farmers have become interested in short straw genotypes which can support higher levels of fertilization and other agronomic practices without lodging. The development of advanced mutant lines from the cultivar IAPAR 3-ARACATU was the main objective of the present work. Preliminary tests with seven different doses of gamma rays were conducted to select the two basic treatments of 37.5 and 42.5 krad (375 and 425 Gy). One kilogram of seeds was treated for each dose to form the two populations: Mutara 1 and Mutara 2. The generations after $M_2$ were managed by three different breeding methods: pedigree, modified pedigree, and modified bulk. In the evaluation of the derived lines, the following parameters were considered: height of the plants, resistance to leaf and stem rust, resistance to leaf blotch, cycle, and grain yield. The largest number of mutant lines was obtained with the pedigree method. The results indicated a higher variability for grain yield than for height reduction and are in accordance with the findings of others. Some promising mutant lines obtained in the present work will be included in yield trials conducted across the Parana state and depending on their performance might be recommended for cultivation or used in future crosses.

## 1. INTRODUCTION

The state of Parana is the major wheat producer of Brazil, contributing over 50% of the national production. The mean yield average has varied from 781 to 2027 kg/ha. Among the factors which contribute to reduced yields and instability of production are the occurrence of adverse climatic factors, high incidence of fungal diseases, soils with high levels of aluminum and inadequate management of the crop. Contributing also to the production instability are the climatic variations that occur because of the location of the state in a large transition zone, and the limited use of new, improved, adapted cultivars as a recommended agronomic practice.

In the decade of the 70's, a wheat breeding programme was initiated in the state, having as major objectives the development of cultivars with high yield potential and stability of production, disease resistance, and acceptable industrial quality.

In 1981 the Instituto Agronomico do Parana-IAPAR released the cultivar IAPAR 3-ARACATU. This cultivar has a yield potential of about 3000 kg/ha and good resistance to leaf and stem rusts (*Puccinia graminis* f. sp. *tritici* and *Puccinia recondita* f. sp. *tritici*). However, because it is a tall cultivar, it was not widely accepted by the farmers in spite of its good agronomic characteristics and production stability. To solve this problem, a breeding project with the objective of developing short genotypes was started in 1986 at Instituto Agronomico do Parana (IAPAR) in cooperation with the Centro de Energia Nuclear na Agricultura (CENA).

Mutation induction has been used effectively in the breeding of cereals to obtain reductions in plant height and lodging resistance [1-4]. It has permitted the selection of advanced lines or

cultivars with reduced height and desirable agronomic characteristics in less time than the conventional breeding methods [4,5]. It has been observed that the incorporation of major genes for height reduction derived from Norin 10 and Tom Thumb into locally adapted cultivars can also negatively affect yield stability and agronomic characteristics of interest [1,6,7]. The development of shorter mutants with desirable agronomic characteristics and yield stability from the cultivar IAPAR 3-ARACATU was the major objective of the present work.

## 2. MATERIALS AND METHODS

*Determination of sensitivity to gamma rays*

Seeds of cultivar IAPAR 3-ARACATU (*Triticum aestivum* L.) were submitted to gamma radiation at the $^{60}Co$ source of CENA-USP in Piracicaba-SP. To determine the sensitivity of the material, seeds (12% moisture) were irradiated with doses of 15, 20, 25, 30, 35, 40 and 45 krad, using the dosage rate of 128 krad/hour. Taking into consideration the height, survival rate, and seedling development at 20 days after sowing, two doses were selected for the definitive treatment: 37.5 and 42.5 krad.

*Obtaining the $M_1$ and successive generations of selfing*

In 1986, one kilogram (approximately 28 000) seeds was irradiated with each dose to obtain two populations, Mutara 1 and Mutara 2, which were sown in the IAPAR Experiment Station at Londrina, together with the control, to obtain the $M_1$ generation. The $M_1$ generation was harvested in bulk, with a sample of two kilograms of seeds (approximately 84 000 seeds) being taken from each dose. In 1987, the seeds were sown in the field together with a control, to obtain the $M_2$ generation. In the $M_3$, $M_4$ and $M_5$ generations, three different selection methods were used: pedigree, modified pedigree, and modified bulk [8]. The pedigree method followed the usual scheme, with the first selection being done in $M_2$ and the progeny of each selected plant being evaluated in the subsequent generation. The modified pedigree method consisted of selecting and bulk harvesting 20 heads within the best progenies of the individual plants selected in the $M_2$ generation. This procedure was followed until the $M_4$ generation.

The modified bulk consisted of an intermediate procedure between the pedigree and bulk. Selection was started in the $M_2$ generation by the elimination of undesirable plants and bulking one head from each of the remaining plants. This procedure was followed until the $M_4$ generation, where individual plant selection was performed.

In the $M_5$ generation, the best advanced mutant lines selected by the pedigree and modified pedigree methods were evaluated in yield trials. The mutant lines obtained by the modified bulk method were evaluated in the $M_6$ generation. The experimental design used was the augmented design, with plots of 6 rows x 5 m, with a spacing of 17 cm between rows, and a seed density of 400 per square meter, similar to the farmers' method of cultivation.

The parameters considered in the evaluations were plant height, resistance to leaf and stem rust, resistance to leaf blotch caused by *Helminthosporium* sp., growth cycle, and grain yield. Plant height was measured on the principal tiller, from the soil surface to the tip of the spike, excluding the awns. The growth cycle was considered to be the number of days from emergence to heading. For the evaluation of leaf and stem rusts, the modified Cobb scale was used [9]. For leaf blotch the scale used was that proposed by Saari and Prescott in 1965 [10].

## 3. RESULTS AND DISCUSSION

*Determination of sensitivity to gamma rays*

The seedling height of the $M_1$ generation and the control, measured 20 days after sowing, is presented in Table I. As expected, a reduction in the height of the plants as a function of the increase in the dose rate was observed. Based on these results, two doses were chosen: 37.5 and 42.5 krad. The first dose caused a reduction of 20 to 25% in the height of the seedlings, compared to the control, while the second caused a more marked reduction, approximately 25 to 35%.

TABLE I. HEIGHT OF CULTIVAR IAPAR 3-ARACATU $M_1$ SEEDLINGS FOLLOWING SEED IRRADIATION WITH GAMMA RAYS.

| Dose (krad) | Height in % of control* |
|---|---|
| 0.0 | 100.0 |
| 15.0 | 101.7 |
| 20.0 | 97.8 |
| 25.0 | 91.7 |
| 30.0 | 87.8 |
| 35.0 | 80.0 |
| 40.0 | 75.0 |
| 45.0 | 65.0 |

* Mean of 4 replications measured 20 days after sowing.

*Results of the selections in the $M_2$ and successive generations of selfing.*

The results obtained from selections in the two populations, Mutara 1 and Mutara 2, with the different breeding methods are presented in Table II. Differences were verified among the numbers of mutant lines obtained from the two populations and from the different selection methods employed.

Comparing the effectiveness of the three selection methods, the greatest number of mutant lines was obtained with the pedigree method. The larger number with the pedigree method may be due to the relatively greater amount of time that must be spent in the populations in the different generations with this method. However, a greater number of mutant lines per se is not advantageous if it is not associated with an effective genetic gain by the selection of lines having reduced height associated with good agronomic characteristics.

Table II shows that 12 advanced lines were selected and evaluated in yield trials that started in 1990. The results of these evaluations are shown in Tables III, IV and V. The behavior of two mutant lines obtained through selection in remnant seed populations are presented in Table VI.

The results of Table III showed that most of the mutant lines evaluated had a similar level of leaf rust resistance compared to the control. There was an increase in the level of resistance to stem rust. The selected mutants were more susceptible to spot blotch than the parent cultivar. There was low variability in growth cycle and height of the mutant lines, except for two which had an 18% reduction in height. In respect to yield, the mutant lines were similar or superior to the control. The results confirmed that the mutants have agronomic potential, but not as marked a height reduction as expected.

TABLE II. NUMBER OF INDIVIDUAL SELECTIONS IN MUTANT POPULATIONS OF CULTIVAR IAPAR 3-ARACATU MANAGED UNDER THREE BREEDING METHODS, 1987 TO 1990.

| Population and method | Year and generation* | | | |
|---|---|---|---|---|
| | 1987 $M_2$ | 1988 $M_3$ | 1989 $M_4$ | 1990 $M_5$ |
| Mutara 1 | | | | |
| Pedigree | 108(I) | 81(I) | 2(L) | Y. trial |
| Modified pedigree | 108(I) | 11(L) | 2(L) | Y. trial |
| Modified bulk | - | - | 28(I) | 2(L) |
| Mutara 2 | | | | |
| Pedigree | 124(I) | 72(I) | 5(L) | Y. trial |
| Modified pedigree | 124(I) | 4(L) | - | - |
| Modified bulk | - | - | 26(I) | 1(L) |

* I=individual plants, L=lines, Y. trial = yield trial.

TABLE III. PERFORMANCE OF SEVEN $M_5$ MUTANT LINES SELECTED BY THE PEDIGREE METHOD FROM THE CULTIVAR IAPAR 3-ARACATU, 1990.

| Genotype | Leaf rust | Stem rust | Leaf blotch | Heading (No. days) | Plant height (m) | Yield kg/ha |
|---|---|---|---|---|---|---|
| Mutara 2-73L-8L-0L | tMS | 5MS | 3 | 67 | 0.90 | 3200 |
| Mutara 2-74L-1L-0L | 10S | 0 | 3 | 68 | 0.90 | 3200 |
| Mutara 1-1L-1L-0L | 0 | 0 | 3 | 65 | 1.10 | 2700 |
| Mutara 1-9L-3L-0L | tS | 0 | 3 | 65 | 1.10 | 2800 |
| Mutara 2-23L-1L-0L | 0 | 0 | 5 | 66 | 1.00 | 2400 |
| Mutara 2-73L-6L-0L | 0 | 0 | 5 | 68 | 1.00 | 3200 |
| Mutara 2-23L-2L-0L | tS | 0 | 5 | 68 | 1.00 | 2560 |
| IAPAR 3-ARACATU(C) | 0 | tS | 1 | 65 | 1.10 | 2500 |

(C)=Control.

The performance of two mutant lines obtained by the modified pedigree method is shown in Table IV. There was no genetic gain related to the reduction in plant height. The mutant lines were similar to the control with regard to disease resistance except for stem rust, in which they showed a significant increase in resistance. Additionally, there was a reduction in growth cycle and grain yield. The low grain yield may be related to an environmental effect.

The results with the three mutant lines obtained through the modified bulk method are presented in Table V. There were no differences for disease reaction and growth cycle, however there was variation in the height of the plants. Two mutant lines showed a 22 and 27% height reduction, when compared to the control. All the mutants were lower in grain yield than the control.

TABLE IV. PERFORMANCE OF TWO $M_5$ MUTANT LINES SELECTED BY THE MODIFIED PEDIGREE METHOD FROM THE CULTIVAR IAPAR 3-ARACATU, 1990.

| Genotype | Leaf rust | Stem rust | Leaf blotch | Heading (No. days) | Plant height (m) | Yield kg/ha |
|---|---|---|---|---|---|---|
| Mutara 1-59L-0L-0L | tR | 0 | 3 | 65 | 1.05 | 1370 |
| Mutara 1-72L-0L-0L | 0 | 0 | 3 | 65 | 1.10 | 920 |
| IAPAR 3-ARACATU(C) | 0 | 10MS | 3 | 70 | 1.10 | 1270 |

(C) = Control.

TABLE V. PERFORMANCE OF THREE $M_5$ MUTANT LINES SELECTED BY THE MODIFIED BULK METHOD FROM THE CULTIVAR IAPAR 3-ARACATU, 1990.

| Genotype | Leaf rust | Stem rust | Leaf blotch | Heading (No. days) | Plant height (m) | Yield kg/ha |
|---|---|---|---|---|---|---|
| Mutara 1-1L-0L-0 | 0 | 0 | 3 | 51 | 0.80 | 940 |
| Mutara 1-6L-0L-0L | 0 | 0 | 3 | 48 | 1.00 | 2000 |
| Mutara 2-14L-0L-0L | 0 | 0 | 3 | 49 | 0.85 | 1760 |
| IAPAR 3-ARACATU(C) | 0 | tS | 3 | 47 | 1.10 | 2500 |

(C) = Control.

As shown in Table VI, the mutants that originated from the selections in the reserve $M_2$ seeds did not differ from the control for growth cycle and disease resistance. There was a height reduction of about 23% and a strong reduction in grain yield, relative to the control.

TABLE VI. PERFORMANCE OF TWO $M_6$ MUTANT LINES, SELECTED BY THE PEDIGREE METHOD FROM REMNANT $M_2$ GENERATION POPULATIONS OF CULTIVAR IAPAR 3-ARACATU, 1990.

| Genotype | Leaf rust | Stem rust | Leaf blotch | Heading (No. days) | Plant height (m) | Yield kg/ha |
|---|---|---|---|---|---|---|
| Mutara 2-73L-5L-1L | 0 | 0 | 3 | 52 | 0.85 | 1960 |
| Mutara 2-73L-5L-2L | 0 | 0 | 3 | 52 | 0.80 | 1500 |
| IAPAR 3-ARACATU (C) | 0 | tS | 3 | 50 | 1.05 | 2700 |

(C) = Control.

The results presented indicated that the mutant lines showed higher variability for grain yield than for plant height in relation to the original cultivar. High average grain yields were observed in three mutant lines selected from the Mutara 2 population. Also, a tendency for greater height reduction among the lower yielding lines was observed.

The low yield level exhibited by some of the lines was not due to sensitivity to aluminum, since all experiments were conducted on limed soils. It was also evident that the lower yields were not due to a high disease incidence, considering the similar or superior disease reactions of the mutants in relation to the control.

The results are in accordance with those achieved in other cereal breeding programmes using mutation induction [11,12]. Mutants which possessed resistance to diseases, environmental stresses, and lodging, combined with earliness and higher and stable yields, have been obtained and many of them have become new cultivars and are being used by the farmers [2-4].

Some promising mutant lines obtained in the present programme will be promoted to preliminary yield trials which are conducted at different locations in the state and, depending on their performance, they may be recommended as new cultivars or utilized in crosses. From the present study, it is clear that through mutation induction, it is possible to obtain improved lines with important agronomic characteristics which can contribute to the overall improvement of the wheat crop.

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# EVALUATION OF ANTHER CULTURE METHODS AND ANDROGENIC CAPACITY OF WHEAT MUTANT LINES

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**Abstract**

Several experiments were conducted with the aim of obtaining haploid plants via anther culture of Chilean spring wheat genotypes. The first experiments compared five culture media and the effect of different incubation conditions on anthers of the genotypes Austral-UACH, Carahue-INIA, Dalcahue-INIA and Pavon 76. The low androgenic response obtained in these experiments, with a maximum of 1.78% callus induction, resulted in a change in methodology. In other experiments, the effect of P-4 callus induction medium and 190-2 plant regeneration medium on $M_2$ and $M_3$ mutant lines of As-Baer, Carahue-INIA, Dalcahue-INIA and Pavon 76 were compared. The effect of two sterilization systems was also studied. These experiments gave a maximum of 55.7% callus formation and a variable organogenesis in three genotypes. Dalcahue-INIA irradiated with 20 krad gamma rays (200 Gy) gave a good organogenic response. The percentage of albino plants ranged between 0 and 83.3% of the total regenerants.

## 1. INTRODUCTION

The development of haploid plants through anther culture has been an efficient way to obtain complete homozygosity or the expression of recessive genes. By doubling the number of chromosomes of these haploid individuals, a genetically stable doubled haploid plant which is completely homozygous may be obtained within one year. The accomplishment of the same stability by means of conventional breeding methods takes a much longer time [1].

Several authors point out that the disadvantage of anther culture is the fact that only a low percentage of anthers gives development of whole plants [2,3]. However, in the past few years the technology of embryogenic callus induction starting from microspores or anthers of several cereal crops has been improved significantly by the use of more efficient culture media [4-10].

The effect of culture temperature as well as the pretreatment of spikes or anthers by cold shock has been studied in detail by several workers [11-14]. The effect of the physical environment of the anther upon callus formation and regeneration of plant has also been studied [11,15]. A key factor of *in vitro* androgenesis is the genotype of the donor plant [3,7,16-18]. Reviews of these and other factors which influence the androgenic response in anther culture have been published by several authors [10,19,20].

The need to improve the existing spring wheat germplasm for south Chilean conditions, especially by means of mutation breeding for tolerance to edaphic stress and other goals [21], led to the implementation of anther culture techniques in order to obtain haploid plants, taking into account the above mentioned success factors.

## 2. MATERIALS AND METHODS

The studies of haploid plant induction of Chilean spring wheat genotypes were carried out in two series of experiments which included different plant materials cultured on distinct nutrient media.

The first series of experiments included three genotypes: Austral-UACH, Dalcahue-INIA, and Carahue-INIA, with Pavon 76 used as a control cultivar because of its good androgenic capacity. Spikes of these genotypes were sterilized with 10% $Ca(ClO)_2$ for 10 minutes. Anthers from the central part of the spikes were removed and plated on 100 x 20 mm petri dishes with 10 ml of solid medium, plating 20 anthers per dish. Five culture media were evaluated: P-2 medium [5]; P-2 plus glutamine (200 mg/1); modified P-2 (without potato extract plus glutamine); 85-D3 medium [7]; and 85-Dl2 medium [7]. All the media were solidified with gelrite (2,5 g/1).

Prior to plating of the anthers, the spikes received a cold treatment at 4°C during 5-7 days. Half of the plated anthers were incubated in the dark, the other half were plated under fluorescent light (2500 lux, 16 hours). In both cases a temperature of 25°C was maintained. Once callus formation was observed, calli were transferred to 80 x 20 mm culture tubes covered with aluminium foil and containing MS medium supplemented with 0.4 mg/l naphthalene acetic acid, 1.0 mg/l benzylaminopurine and 1.0 mg/l gibberellic acid and incubated under light at the same temperature. Evaluation of callus formation normally was performed 40 days after plating of anthers.

In the second series of experiments the methodology was changed in regard to spike sterilization, culture media used and incubation temperature. These experiments were carried out using Pavon 76 as a control and the genotypes As-Baer (20 krad), Carahue INIA (20 and 30 krad), and Dalcahue-INIA (20 and 30 krad). Materials were $M_2$ and $M_3$ generations of these cultivars which were originally irradiated with 20 and 30 krad (200 and 300 Gy) of gamma rays. The spikes were harvested when their apex was approximately one inch below the second leaf. At this stage, microspores of the anthers taken from the central part of the spike were at mid- to late-uninucleate stage.

As in the first series of experiments, the spikes received a cold shock (4°C) for 5-7 days prior to harvesting the anthers. The sterilization of the spikes was performed by wiping the spikes with a 70% ethanol-soaked cotton ball, or by submerging the spikes for 20 minutes in a 20% NaOCl solution.

The culture medium used in these experiments was a P-4 medium [6], according to the methodology proposed by Zhou and Konzak [3]. This is a liquid medium which contains 1.5 mg/l 2,4-D and 0.5 mg/l kinetin. It is used exclusively for the callus induction phase. For the regeneration of shoots and roots, a 190-2 medium [8] was used, which does not contain phytohormones but includes some amino acids. This medium was gelled with 2.5 g/l gelrite. Incubation during the callus induction phase was carried out in the dark with a temperature of 26-28 $\pm$ 2°C. Regeneration was performed at the same temperature but under light (2500 lux, 16 hours).

## 3. RESULTS AND DISCUSSION

Generally, the results of the first series of experiments (Table I) indicated poor callus development, which did not exceed 2.9% of the plated anthers at best. It is clear that from the media used, the formulations proposed by Liang and coworkers [7] show a better androgenic response as a mean of the different genotypes and incubation conditions studied.

Although the media 85-D3 and 85-Dl2 were originally developed for direct generation of plants, the obtained calli did not appear to be embryogenic. That is why there was no development of plants, contrasting with the results obtained by Liang *et al.* [7]. The rate of callus development obtained in other studies carried out in Chile [18] with the 85-D3 medium did not exceed 1.09% of plant-producing calli which, nevertheless, developed some plants.

TABLE I. EFFECT OF CULTURE MEDIA, TYPE OF INCUBATION, AND GENOTYPE ON CALLUS INDUCTION.

| Treatment | Anthers | | |
|---|---|---|---|
| | No. plated | No. with calli | % with calli |
| Medium | | | |
| P-2 4250 | 2 | 0.05 | |
| P-2 + glutamine | 4195 | 8 | 0.19 |
| P-2 mod.* + glutamine | 4400 | 8 | 0.18 |
| 85-D3 1262 | 8 | 0.63 | |
| 85-DI2 1262 | 37 | 2.93 | |
| Incubation | | | |
| Light 7944 | 20 | 0.25 | |
| Dark 7425 | 43 | 0.58 | |
| Genotype | | | |
| Austral-UACH | 4955 | 6 | 0.12 |
| Dalcahue-INIA | 3440 | 0 | 0.00 |
| Carahue-INIA | 4450 | 12 | 0.27 |
| Pavón 76 | 2524 | 45 | 1.78 |
| TOTAL/MEAN | 15 369 | 63 | 0.41 |

* Without potato extract.

In the same study [18], the use of P-2 medium gave rise to 1.83% of the anthers producing calli, contrasting with our results where only 0.05% of the anthers developed calli. Nevertheless, these results were improved by the addition of glutamine, which seems to be a key factor in barley anther culture [23].

The incubation conditions of these experiments show that a higher percentage of callus development was achieved in anthers incubated in the dark, as compared to those kept constantly under light conditions. These results agree with other works [3]. However, previous studies [11,16] did not show a clear effect of the light factor upon the degree of the androgenic response of the genotypes.

In the same way, the genotypes used in this series of experiments also showed a wide range of callus induction capacity. In Pavon 76, the best genotype, only 1.78% of the anthers formed calli. This contrasts with Dalcahue -INIA, which did not show any ability to form calli under the conditions of these experiments. The cultivars Austral-UACH and Carahue-INIA showed an intermediate callus development. The use of Pavon 76 as a control variety, well known for its proven androgenic capacity [3,17] and its low response level under our culture conditions, clearly show a deficiency in the system. This may be attributed to the culture medium conditions, since the P-2 medium and its modifications [5] as well as the media by Liang *et al.* [7] have been widely replaced by P-4 medium [12] for callus induction, followed by a differentiation medium [8]. A possible additional reason for the low callus induction may be the use of solid media, since several investigations have been more successful on liquid media [2,3,11] because of reduced dehydration of the anthers.

In the second series of experiments, the effect of the different genotypes and distinct spike - sterilization methods upon the callus formation rate of the anthers was investigated. The response of the genotypes to anther culture may be seen in Table II, where the three genotypes used in the first

series of experiments are shown. The use of another culture medium, as well as the change of the incubation temperature led to a greater success in the callus formation rates. As can be seen, 17.1% of the total anthers of Pavon 76 formed calli, which corresponds to 55.7% of those anthers which remained sterile.

TABLE II. RESPONSE OF SEVERAL SPRING WHEAT GENOTYPES TO ANTHER CULTURE: NUMBER AND PERCENTAGE OF INDUCED CALLI.

| Treatment | Anthers | | Induced calli (No.) | Calli/anther | |
|---|---|---|---|---|---|
| | Plated (No.) | Sterile (No.) | | Plated (%) | Sterile (%) |
| Pavón 76 | 286 | 88 | 49 | 17.1 | 55.7 |
| As-Baer, 20 krad | 308 | 154 | 2 | 0.6 | 1.3 |
| Carahue-INIA, 20 krad | 220 | 66 | 2 | 0.9 | 3.0 |
| Carahue-INIA, 30 krad | 308 | 154 | 9 | 2.9 | 5.8 |
| Dalcahue-INIA, 20 krad | 308 | 176 | 30 | 9.7 | 17.0 |
| Dalcahue-INIA, 30 krad | 88 | 44 | 2 | 2.3 | 4.5 |
| Total/mean | 1518 | 682 | 94 | 6.2 | 13.8 |

The response of Dalcahue-INIA (20 krad) was outstanding. This cultivar showed no callus formation in the first series of experiments. The mutagenic effect upon callus formation is not clear, because the same cultivar irradiated with 30 krad showed a lower callus formation capacity. The mean of all genotypes was 13.8 anthers with callus formation per 100 sterile anthers. This percentage is similar or even superior to the response found in other investigations [11,16,18], but inferior to the results obtained by Zhou and Konzak [3]. A key factor in this difference may be the variation in incubation temperature, which in this case could not be kept constant because of the use of an incubation room with a maximum fluctuation range of 6°C (night/day) in the summer. In this regard, other investigations [10,13] indicated the importance of the incubation temperature and its dependence on the culture conditions of the donor plant.

The effect of the spike sterilization system may be observed in Table III, which shows that external sterilization with ethanol produced a higher number of sterile anthers as compared to the NaOCl treatment. Nevertheless, callus induction was clearly higher when clorox was used instead of ethanol. This might be attributable to a higher dehydration of anthers by the use of ethanol. Other workers have successfully used both sterilization systems. Ethanol [2,3] as well as clorox [11,16] have proven to be good spike sterilization chemicals but their effects have not yet been compared.

The subculture of calli on 190-2 medium [8] proved to be efficient for the development of plantlets in some genotypes (Table IV). Nevertheless, the degree of organogenic response was variable, since some genotypes (As-Baer, Carahue-INIA 20 krad and Dalcahue-INIA 30 krad) did not show evidence of the production of embryogenic callus, which was revealed by the formation of a white, friable callus mass which was unable to develop either roots or shoot. From those genotypes which showed organogenesis, Pavon 76 had a higher percentage of root formation. On the other hand, Dalcahue-INIA (20 krad) in 55% of the subculture calli showed root and shoot development. These results indicate a variable organogenic capacity of the genotypes studied. In a previous study [3] using the same regeneration medium, similar results were achieved even though the range in variation among cultivars was lower. Studies carried out with other Chilean genotypes [18] revealed a low plant development rate which did not exceed 0.36% of anthers plated on several culture media.

TABLE III. EFFECT OF SPIKE STERILIZATION TREATMENT ON NUMBER AND PERCENTAGE OF CALLI INDUCED.

| Treatment | Anthers | | Induced calli (No.) | Calli/anther | |
|---|---|---|---|---|---|
| | Plated (No.) | Sterile (No.) | | Plated (%) | Sterile (%) |
| Ethanol 70% | 902 | 462 | 45 | 5.0 | 9.7 |
| NaOC1 20%, 20 min. | 616 | 220 | 49 | 8.0 | 22.3 |
| Total/mean | 1518 | 682 | 94 | 6.2 | 13.8 |

TABLE IV. DEVELOPMENT OF PLANTLETS FROM WHEAT ANTHER CALLI (90 DAYS AFTER PLATING).

| Genotype | Calli transferred (No.) | Growth (%) | | |
|---|---|---|---|---|
| | | Nil | Roots | Roots + shoots |
| Pavon 76 | 55 | 43.6 | 40.0 | 16.4 |
| As-Baer, 20 krad | 2 | 100.0 | 0.0 | 0.0 |
| Carahue-INIA, 20 krad | 4 | 100.0 | 0.0 | 0.0 |
| Carahue-INIA, 30 krad | 9 | 55.6 | 22.2 | 11.2 |
| Dalcahue-INIA, 20 krad | 20 | 25.0 | 20.0 | 55.0 |
| Dalcahue-INIA, 30 krad | 2 | 100.0 | 0.0 | 0.0 |
| Total/mean | 92 | 70.7 | 13.7 | 13.8 |

Some of the developed plantlets were albinos (Table V). This percentage varied among the genotypes. Albinism of the regenerated plants ranged between 0 and 83.3% of all the plants obtained from the embryogenic calli. This proportion of albinism is similar to the values observed previously by other authors on the same regeneration medium [3] as well as on other less efficient media [16,18,24]. The problem of albinism could be due to the metamorphosis of plastids in the transition from the sporophytic to gametophytic phase in which plastids would not be able to develop into chloroplasts. During metamorphosis, a plastid-DNA degradation in some microspores capable of developing multicellular structures might have occurred, giving rise to a certain proportion of albino regenerants [10].

TABLE V. EFFECT OF WHEAT GENOTYPE AND ITS MUTAGENIC TREATMENT ON GREEN AND ALBINO PLANT DEVELOPMENT.

| Genotype | Plant development | | | | |
|---|---|---|---|---|---|
| | Total | Green | | Albino | |
| | No. | No. | % | No. | % |
| Pavon 76 | 18 | 18 | 100 | 0 | 0.0 |
| As-Baer, 20 krad | - | - | - | - | - |
| Carahue-INIA, 20 krad | - | - | - | - | - |
| Carahue-INIA, 30 krad | 6 | 1 | 16.7 | 5 | 83.3 |
| Dalcahue-INIA, 20 krad | 27 | 20 | 74.1 | 7 | 25.9 |
| Dalcahue-INIA, 30 krad | - | - | - | - | - |
| Total/mean | 51 | 39 | 76.5 | 12 | 23.5 |

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# YIELD STABILITY OF WHEAT MUTANT LINES

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**Abstract**

Genotype by environmental interactions and yield stability were estimated for 26 wheat mutant lines and two original parents in regional experiments in Chile's southern region in 1985 - 1989. The magnitude of the G x E interaction was estimated using correlation methods; analysis of yield stability was done using Eberhart and Russel's method. Ten percent of the interaction was attributed to genetic differences in yield among the mutant lines and the remaining 90% was assigned to the lack of correlation among the years. The analysis of yield stability allowed us to define mutant lines according to their sensitivity to environmental influences, their reaction to high and low yield environments and to the consistency of their behaviour through the years and locations. The presence of genetic variability for yield stability indicated that selection could be effective for this character.

## 1. INTRODUCTION

The combined analysis of variance has been the traditional method to investigate interaction of genotypes by environment in experimental groups [1]. With this analysis, the magnitude of the interaction is evaluated, according to the objectives of the breeder, through the variance of the effects of genotypes × locations, genotypes × years, genotypes × locations × years and others. These parameters, although useful, do not provide adequate information about the behaviour of the genotypes or cultivars in the different environments in which they are evaluated [2,3]. Therefore, complementary analyses of partition of variance due to interaction, are needed. A preliminary method to study the nature of the organization of interactions is to apply the correlation method [4], where interaction is broken down into two parts. One part of it is due to the difference in genetic variability within the environments, while the other is associated with the absence of a perfect linear correlation among genetic treatments from one environment to another.

Another approach to genotype by environment interaction is the one related to adaptation and stability of genotypes or cultivars. Although related, adaptation and stability are different phenomena. Mariotti *et al.* [6] use the term adaptability to refer to the potential capacity of genotypes to respond advantageously to environmental stimuli, an advantage from the agricultural point of view. In turn, stability is defined as the capacity of genotypes to show the most constant performance possible, in relation to variations of environmental quality.

At present, several investigators [4] prefer to use the term adaptability to refer to ecological adaptation to different environments such as sites or other geographic conditions (geographic stability). Therefore, stability is used to refer to the greater or lesser ability of the genotype to adapt to climatic conditions across agricultural years within a given ecological zone or site (temporal stability). The former is, in fact, the one that most interests the farmer. Several methods have been proposed to study stability [5,7-13]. The differences between them stem from the concepts of stability and from the biometric methods used to estimate it.

To complement the conventional breeding methods for resistance to diseases, tolerance to edaphic stresses, protein content and grain yield, mutation techniques were incorporated into the Wheat Breeding Programme of the Universidad Austral de Chile, and extended in 1981 and 1987.

The main results obtained from these studies have been presented by Barriga and Fuentes [14, 15] and Barriga *et al.* [16-21]. However, the yield stability of these mutants, a critical parameter in the development of superior cultivars, especially in highly variable environments, has not been studied.

In view of the above, the objective of this paper is to analyze genotype × environment interaction and to estimate the temporal stability of yield of mutant lines of wheat during a five-year period in two localities of the Lake Region in Chile (latitude 39° - 44° South).

## 2. MATERIAL AND METHODS

The analyzed germplasm consisted of twenty-six promising mutants of spring wheat and their parent cultivars (Austral and Huenufén) from the collection of mutant lines of the Wheat Improvement Programme. Evaluation of their performance was carried out in two different agricultural areas of the Lake Region in Chile under different management procedures during a five-year period (1984/85 to 1989/90). One of the areas was located in Valdivia, at the Santa Rosa Experimental Station, while the other was in San Juan de la Costa, Osorno, at the Quimei Experimental Sub-station.

The experimental design used was a complete randomized block with four replications in plots of five 2 m rows spaced 0.20 m apart. Fertilization at the Santa Rosa Station was 96, 200 and 50 kg/ha of N, $P_2O_5$ and $K_2O$ respectively; at the Quimei Sub-station, it was 12.8 and 36.8 kg/ha of N and $P_2O_5$ respectively, without subsequent weed control, simulating the management of the San Juan de la Costa area.

Initially, the values obtained for yield were subject to a combined analysis of variance of groups for experiments by locations. Later, the correlation method [4] was used to analyze the magnitude of the genotype-environment interaction for yield by location for five years, according to the following equation:

$$s_{GE}^2 = \sum_{j<j'}^{1} [(s_{G_j} - s_{G_j}^2) + 2(1 - r_{jj'}) s_{G_j} . s_{G_{j'}}]$$

where $s_{GE}^2$ is the genotype interaction variance x environment; $s_{Gj}$ is the genetic standard deviation of the character in the environment j; and $r_{jj'}$, is the correlation between j and j' for a given character.

As can be seen, the equation is formed of two parts: one, due to the difference in the genetic variability within the environment, represented by ($s_{Gj}$ - $s_{Gj'}$), and the other is due to the lack of a perfect linear correlation between genetic treatments, from one environment to another: a component implicit in the term

$$(1 - r_{jj'}) s_{G_j} . s_{G_{j'}}$$

Temporal stability estimates for yield of the mutant lines of wheat were obtained using the simple linear regression method proposed by Eberhart and Russel [8]. According to the model, each genotype is characterized by two stability parameters: the regression coefficient (b) which predicts

stability of the genotype to changing environments, and the deviation of the regression ($s_d$), which provides evidence of the repeatability of performance.

## 3. RESULTS AND DISCUSSION

The results of the combined analysis of variance (Table I) show significant differences in the yield of the mutant lines used for this study in the two locations. Variability from one year to the next was sufficiently high to produce significant genotypes × environments interactions.

TABLE I. COMBINED ANALYSIS OF VARIANCE FOR YIELD OF WHEAT GENOTYPES OVER 5 YEARS AT 2 LOCATIONS.

| Source of variation | df | Mean square | |
|---|---|---|---|
| | | Santa Rosa | Quimei |
| Genotypes (G) | 27 | 673 908.3 ** | 693 294.9 ** |
| Environments (E) | 4 | 6 042 943.3 ** | 6 360 743.5 ** |
| G X E | 108 | 208 468.3 ** | 132 049.5 * |
| Mean error | 405 | 75 027.1 | 106 812.5 |

* Significant at the 5% level. ** Significant at the 1% level.

When the magnitude of the interaction is analyzed (Table II), one can see that approximately 10% is attributable to differences in genetic variability of the yield of the mutant lines within years; 90% is associated with the lack of correlation among mutant lines from one year to the other. It is important to point out that the problem lies in the second part of the interaction, since a low correlation means that the superior mutant lines in one environment may not behave the same way in another. Equally important is the fact that there can be interaction even when there is a high correlation but, in that case, in spite of the interaction, there would be no difficulty in selecting superior lines.

TABLE II. MAGNITUDE AND PARTITION OF GENOTYPE × ENVIRONMENT INTERACTION FOR YIELD OF TWENTY-EIGHT WHEAT GENOTYPES OVER A FIVE YEAR PERIOD AT TWO LOCATIONS.

| Parameter | Santa Rosa | | Quimei | |
|---|---|---|---|---|
| | Absolute value | % | Absolute value | % |
| $s_{GE}^2$ | 208,468.2 | 100.0 | 132,049.5 | 100.00 |
| $(s_{G_j} - s_{G_{j'}})$ | 18,809.0 | 9.0 | 12,912.1 | 9.8 |
| $(1-r_{jj'})\ s_{G_j} \cdot s_{G_{j'}}$ | 189,658.7 | 91.0 | 119,137.4 | 90.2 |

The stability analysis (Table III), according to Eberhart and Russel's model [8], shows that the environment was negative for the mean yield of the mutant lines during the first two years at Santa Rosa, it being proportionally more positive during the 1988/89 agricultural season. On the other hand, in Quimei the influence of the environment was greater, presenting negative indices for the first, second and fifth year and highly positive results for the third year of evaluation.

TABLE III. ENVIRONMENTAL INDICES ($I_j$) IN TWO LOCATIONS OVER FIVE YEARS.

| Year | Location | |
|---|---|---|
| | Santa Rosa | Quimei |
| 1985/86 | -598.36 | -795.85 |
| 1986/87 | -380.08 | -248.71 |
| 1987/88 | 383.31 | 958.72 |
| 1988/89 | 433.14 | 642.58 |
| 1989/90 | 161.99 | -556.74 |

It is interesting to note that, under advanced technology, i.e. high fertilization and weed control, the environment plays a less important role than under deficient conditions where any environmental change affects yield considerably.

In regard to the stability parameters of the mutant lines, we can see that in Santa Rosa (Table IV) the M-01 and M-19 mutant lines showed yields significantly higher (13%) than the mean of the controls. Their mean regression coefficients were close to one and deviations from regression were comparatively low. These characteristics define them as mutant lines of high productivity, with a capacity to express their genetic yield potential uniformly and in a predictable manner under different environmental conditions, with the use of advanced technology.

At Quimei (Table IV), the outstanding mutant lines were M-09, M-16 and M-20 since they had a 30% mean yield increase relative to the mean of the controls. However, their regression coefficients were higher than one and their deviations from the regression were high. These lines can be defined as productive, highly sensitive to environmental changes, and with the ability to make better use than other mutant lines of the advantages offered by the best environments, under marginal agricultural conditions.

The results indicate that adaptability and stability are not correlated, even when they may have different genetic bases. In practical terms, selection for greater adaptability (geographic stability) does not guarantee stability in response to climatic fluctuations across years (temporal stability).

If we analyze the genetic stability of the mutant lines in both localities (Table IV), we can see that the only line that produced a highly satisfactory yield, with a mean regression coefficient (b) close to one and comparatively lower regression deviations ($s_d$), was M-19.

The results indicate that M-19 has the ability to produce predictable, stable yields under a wide range of environmental conditions, independent of the production system. It is a line which may be highly recommended for the southern zone of the country, since it would assure an adequate and stable

production. Because of its superior characteristics, it is included in the National Cooperative Wheat Programme for Yield.

TABLE IV. GENOTYPE MEAN YIELD, REGRESSION COEFFICIENT, DEVIATION FROM REGRESSION AND DETERMINATION COEFFICIENT IN SANTA ROSA AND QUIMEI.

| Genotype | Santa Rosa | | | | Quimei | | | |
|---|---|---|---|---|---|---|---|---|
| | Mean yield (kg/ha) | Regression coefficient (bi) | Deviation from regression ($s_{di}$) (kg/ha) | Determination coefficient ($R^2$) | Mean yield (kg/ha) | Regression coefficient (bi) | Deviation from regression ($s_{di}$) (kg/ha) | Determination coefficient ($R^2$) |
| M-01 | 5069 | 1.137 | 84 | 0.819 | 2152 | 1.156 | 255 | 0.961 |
| M-02 | 4857 | 1.143 | 205 | 0.919 | 2143 | 1.016 | 138 | 0.902 |
| M-03 | 4865 | 1.023 | 253 | 0.964 | 2324 | 1.198 | 147 | 0.929 |
| M-04 | 4673 | 0.411 | 373 | 0.293 | 2267 | 1.080 | 223 | 0.853 |
| M-05 | 4533 | 0.450 | 177 | 0.354 | 2371 | 1.092 | 125 | 0.883 |
| M-06 | 4246 | 0.730 | 338 | 0.448 | 2269 | 1.090 | 332 | 0.810 |
| M-07 | 4896 | 0.959 | 166 | 0.721 | 2201 | 1.231 | 62 | 0.920 |
| M-08 | 4321 | 1.035 | 295 | 0.655 | 1901 | 1.031 | 311 | 0.988 |
| M-09 | 4506 | 0.631 | 305 | 0.406 | 2746 | 1.476 | 30 | 0.941 |
| M-10 | 4330 | 0.737 | 131 | 0.729 | 2042 | 0.865 | 181 | 0.807 |
| Austral | 5119 | 0.454 | 144 | 0.522 | 2616 | 1.152 | 273 | 0.970 |
| M-11 | 4817 | 1.211 | 31 | 0.847 | 2207 | 1.336 | 272 | 0.885 |
| M-12 | 4477 | 0.700 | 67 | 0.667 | 2019 | 0.684 | 218 | 0.703 |
| M-13 | 3740 | 1.651 | 314 | 0.819 | 2370 | 1.667 | 460 | 0.976 |
| M-14 | 3952 | 1.632 | 286 | 0.830 | 1422 | 0.460 | 26 | 0.766 |
| M-15 | 4198 | 2.463 | 172 | 0.943 | 1960 | 0.772 | 258 | 0.988 |
| M-16 | 4664 | 0.983 | 245 | 0.949 | 2996 | 1.001 | 284 | 0.781 |
| M-17 | 4952 | 1.180 | 245 | 0.964 | 2581 | 1.140 | 252 | 0.983 |
| M-18 | 4443 | 0.964 | 648 | 0.351 | 2509 | 0.830 | 277 | 0.780 |
| M-19 | 5216 | 0.965 | 126 | 0.747 | 2632 | 0.991 | 190 | 0.864 |
| M-20 | 4903 | 0.829 | 87 | 0.705 | 2761 | 1.477 | 387 | 0.885 |
| Huenufén | 3974 | 1.427 | 68 | 0.880 | 1761 | 0.744 | 492 | 0.743 |
| M-21 | 4424 | 1.106 | 339 | 0.650 | 2180 | 0.906 | 279 | 0.746 |
| M-22 | 4434 | 1.451 | 276 | 0.800 | 2598 | 1.185 | 341 | 0.907 |
| M-23 | 4435 | 0.881 | 282 | 0.592 | 1491 | 0.231 | 274 | 0.234 |
| M-24 | 4144 | 2.363 | 1142 | 0.538 | 1766 | 0.507 | 157 | 0.920 |
| M-25 | 4521 | 0.104 | 438 | 0.012 | 2224 | 0.946 | 44 | 0.830 |
| M-26 | 4665 | 0.213 | 209 | 0.099 | 2215 | 0.745 | 221 | 0.882 |

The often observed association between yield and stability [22,23] shows that stability is heritable as reported also by Vencovsky and Torres [5] in corn. They noted that the degree of genetic determination of stability is lower than that of yield. In view of these results, it can be concluded that it is possible to obtain genetic improvement for temporal stability in wheat, but would be more difficult to accomplish than for yield.

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# DOUBLED HAPLOIDS AND MUTAGENESIS IN WHEAT: IMPROVEMENT OF ANTHER CULTURE AND CHROMOSOME DOUBLING TECHNIQUES

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**Abstract**

A reliable anther culture technique that could be used by wheat breeders in their programmes for the routine production of androgenic plantlets was established. This, together with mutagenic techniques, could broaden the genetic variability and accelerate cultivar development in this species.

## 1. INTRODUCTION

Conventional breeding techniques are usually used in the INIA Wheat Breeding Programme. Mutagenesis and anther culture have not been used in spite of the fact that genetic variability can be increased and the time required to develop new cultivars shortened. Anther culture use is limited in wheat because few plantlets can be obtained and androgenic capacity is highly dependent on genotypes. A series of experiments was conducted to evaluate the androgenic ability of Chilean wheat germplasm and to improve the anther culture and colchicine doubling techniques.

## 2. EXPERIMENTAL METHODS AND RESULTS

A series of experiments was conducted during 3 seasons in order to evaluate genotype response, different culture media and various haploidization techniques. In the first experiment the androgenic ability of 19 cultivars was evaluated on 5 culture media, with and without a cold pretreatment (4°C for 3-7 days), osmotic pressure (Mannitol 0.8 M for 1 hr) and incubation under darkness (10 days). In the second experiment, morphological characteristics of the spike were related to pollen development at the time of anther extraction. The effect of 2 gametocides (Hybrex and Ethrel), applied to immature spikes prior to meiosis, on the androgenic capacity was determined. The effect of ANA (1 and 2 mg/l) and 2,4-D (2-3 mg/l) in the presence of kinetin (1.5 mg/1) was also evaluated in another experiment. Finally, anther culture media $N_6$ and Potato-4 were compared in their liquid or solid forms with and without Ficoll-400 in the medium. Experiments were incubated at 25±2°C with 50 $\mu$E $m^{-2}sec^{-1}$ irradiation and 16 hrs of light. After 1 month in this condition, the calli were transferred to a regenerative medium, after which plants were acclimated in a greenhouse. Chromosomal doubling was done using colchicine (0.2%) applied *in vitro* prior to acclimation or *in vivo* to acclimated plants prior to shoot elongation.

The various experiments showed the following results:

1. Genotypes differed in their androgenic capacity, as expected.

2. Cold pre-treatment and dark incubation showed favorable effects on androgenesis, but application of osmotic pressure had no effect.

3. Anther extraction should be done when the apex of the spike reaches 3/4 of the distance between the base of the sheath of the second leaf and the ligule of the flag leaf.

4. Hybrex increased androgenic response in 2 out of 30 times. Ethrel showed a less clear effect.

5. 2,4-D at 2 mg/l plus kinetin at 1.5 mg/l were the two growth regulators that increased the androgenic response best.

6. Solid Potato-4 medium increased callus formation and plant regeneration as compared to the liquid form; however, the opposite was true for $N_6$ medium, where the liquid form resulted in increased plant regeneration and fewer albino plantlets. This same effect was observed when the $N_6$ medium was used together with Ficoll-400.

7. Chromosome doubling using colchicine was more effective when the treatment was given after the plantlets were fully acclimated.

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# MUTATION BREEDING OF BARLEY IN PERU

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**Abstract**

Barley has special value as a food for people living in the Andes mountain area of Peru. It is also the basic raw material for the malting industry. The factors limiting barley production in Peru are mainly diseases, low soil fertility, low rainfall, drought, frost and hail.

Improved varieties should have the capability to overcome most of the factors limiting production. To achieve this aim, it is necessary to apply appropriate methods in mutation induction,and crossing and selection. The mutation induction methods are used to improve well adapted varieties and lines. The barley variety Buenavista is suited to the Altiplano conditions. Because of its spikes, it can withstand hail damage better than other varieties. Naked barley is appreciated very much as a different type of food by people living in the highlands. Based on these facts, a mutation breeding programme was initiated to search for naked kernels and other interesting mutants in the Buenavista variety.

The mutagenic agents applied were: gamma rays at 20 and 30 krad (200 and 300 Gy); EMS at 0.05 M and 0.1 M; and sodium azide at 0.001 and 0.004 M. The $M_1$ populations were planted in Callejon de Huaylas at 2700 m of elevation. The $M_2$ generation was grown at La Molina on the campus of the University at 200 m of elevation. The total number of plants in the $M_2$ was 187 574. The frequency of chlorophyll mutations ranged from 1.13% to 5.94%. The highest doses of mutagens resulted in a higher number of chlorophyll mutations, while in the case of naked kernels selected from the bulked seeds of each treatment, the lower dose gave the higher number. The frequency of mutants with naked kernels ranged from 0.07% to 0.12%.

The main goal of obtaining naked kernel mutants was achieved. At the applied treatments gamma rays were more effective as a mutagenic agent for this purpose than EMS or sodium azide.

## 1. INTRODUCTION

Barley is an important crop in the highlands of Peru. It is used as a food in the Andes mountain areas and also to some extent in coastal cities. The total production is about 150 000 t/year, from which about 20% goes to the malting industry. Imports of grain and malt are about 80 000 t/year. The Universidad Nacional Agraria La Molina through its Cereals Programme has released several barley varieties, such as Zapata, UNA 80, UNA 8270, Yanamuclo, and recently, Buenavista, which cover about 70% of the total barley growing area. The main problems with barley are susceptibility to several diseases, such as rusts, spot blotch, scald, powdery mildew and barley yellow dwarf virus (BYDV). Other problems are the yield-limiting effects of drought, frost, hail, low rainfall, and low soil fertility. Grain quality for both food and malt is approaching good levels with the variety Yanamuclo, whose quality is similar to that of imported barley.

During the last five years several approaches have been tried in order to obtain through mutations new genetic variation useful for breeding purposes as well as possible new varieties. In several experiments conducted between 1986-1989 on induced mutations in barley, only the Buenavista variety has shown marked mutation rates.

## 2. MATERIALS AND METHODS

*Varieties*

The varieties used are well adapted genotypes that lack some important traits. They are as follows:

| *Parent lines* | *Objective of mutation breeding* |
|---|---|
| Period 1986-1989 | |
| Breun's Wisa/UNA 392 | Resistance to *Puccinia hordei* |
| Breun's Wisa/UNA 8273 | " |
| Mona Emir/Bco. Gva/Abyssinian | " |
| Mazurka/Mullers Heydla | " |
| Yanamuclo | " |
| Lignee 640 | Light color of grain |
| Period 1990-91 | |
| Buenavista | Naked kernels and other useful mutants |

*Mutagenic agents*

a. Gamma rays.
b. EMS.
c. Sodium azide.

*Experimental sites*

La Molina: Central coast, 200 m elevation.
Callejon de Huaylas: Northern Sierra, 2700 m elevation.

*Handling of mutated populations*

$M_1$ The number of treated seeds ranged from 5000 to 10 000. Seeding rate was normal and rows of the parent variety were included. The populations were generally isolated by space or living barriers. Harvesting was usually done on the basis of individual heads. Percent germination and plant survival were recorded.

$M_2$ The seeds of this generation were planted as head rows. Rows of untreated parents were included as controls. The observations recorded included: frequency of chlorophyll mutations, heading and maturity date, disease reaction, and morphological characters of plants and grains (in the laboratory). The putative mutants were selected on an individual plant basis. From rows in which chlorophyll mutations were observed, samples of heads of normal plants were harvested as probable heterozygotes that would segregate in the $M_3$ generation.

$M_3$ Comprised of progenies of mutants selected in $M_2$. Samples collected in $M_2$ chlorophyll mutant rows. Bulk selection of mutations for some morphological grain traits.

$M_4$ Seed increase of mutants, and preliminary yield trial.

$M_5$ Selected mutants were included in yield trials of advanced lines at different locations.

## 3. RESULTS AND DISCUSSION

*Summary of 1986-89 period*

In this period the number of useful mutants was very low due to the uneven effect of the radiation unit. The selected mutants were as follows:

| *Genotypes* | *Selected mutants* |
|---|---|
| Breun's Wisa/UNA 392 | (a) Early |
| Breun's Wisa/UNA 8723 | (a) Early |
| Brevia/UNA 8309 | (a) Early |
| Carina/UNA 8270 | (a) Early |
| Lignee 640 | (a) Early |
| | (b) Long spike |
| | (c) Vigorous plant |
| | (d) Lightly coloured grain |

Earliness and changes in the spike were observed in most cases. Yield trials showed no advantage over the parent varieties or advanced lines.

*Summary of 1990-91 period*

A new barley variety, Buenavista, which was selected from a cross made at CIMMYT (P7 1318-Row 134.73 CMB.78A415-3An-Lm-An) was released for the high plateau of Puno. Because of its drooping spike, it is damaged less by hail than the other varieties grown in the area.

The common covered grain is widely used but naked seeded varieties are best suited for some types of food. Improved varieties with naked kernels are not available. Therefore, the main goal of this project was to search for a naked grain mutant as an alternative to hulled grain varieties.

*$M_1$ generation*

Table I shows the effects of the mutagenic treatments on emergence and seedling height. The high dose of each treatment reduced the percent emergence the most. The most severe reduction in emergence was caused by the 30 krad gamma rays treatment.

*$M_2$ generation*

*Chlorophyll mutations*

Numerous chlorophyll mutations were recorded. Wide variability was observed in the chemical mutagenic treatments. The mutant types included albina, xantha, alboxantha, xanthoalba, viridoalbina, viridoxantha, xanthoviridis, virescens, chlorina, lutescens, albescens, tigrina, striata and maculata.

TABLE I. EFFECT OF MUTAGENIC TREATMENTS ON EMERGENCE AND SEEDLING HEIGHT IN THE $M_1$ GENERATION OF BUENAVISTA BARLEY, CALLEJON DE HUYLAS, 1990-91.

| Mutagenic agents | Dose/ concentration | Emergence (%) | Seedling height (% of control) |
|---|---|---|---|
| Gamma rays | 20 krad | 75.89 | 88.3 |
| | 30 krad | 35.74 | 79.4 |
| EMS | 0.05 M | 83.90 | 90.4 |
| | 0.10 M | 54.65 | 77.6 |
| Sodium azide | 0.001 M | 95.65 | 99.6 |
| | 0.004 M | 74.54 | 86.5 |
| Control | - | 100.00 | 100.0 |

The highest dose of each mutagen treatment produced the highest frequency of chlorophyll mutants (Table II). Among the chemical mutagenic agents, EMS at 0.1 M and sodium azide at 0.004 M had mutation frequencies of 5.63% and 5.94%, respectively. Albino types were the most frequent mutation types caused by gamma rays, at both doses, followed by virescens types at 30 krad and maculata at 20 and 30 krad. The treatments with EMS followed the same pattern as gamma rays, but high frequencies of tigrina and xantha types were also detected. Sodium azide showed the same high frequency of these mutant types, particularly at the high dose.

TABLE II. FREQUENCY OF CHLOROPHYLL MUTATION TYPES IN THE $M_2$ POPULATION OF BUENAVISTA BARLEY, LA MOLINA, 1990-91.

| Mutations | Gamma rays (krad) | | EMS (M) | | Sodium azide (M) | |
|---|---|---|---|---|---|---|
| | 20 | 30 | 0.05 | 0.10 | 0.001 | 0.004 |
| Albino | 0.6161 | 0.4189 | 0.4320 | 1.4515 | 0.2944 | 1.2018 |
| Xantha | 0.0616 | 0.2514 | 0.1125 | 0.6774 | 0.1385 | 0.4622 |
| Alboxantha | - | - | 0.0049 | 0.0194 | 0.0011 | - |
| Xanthoalbina | 0.0560 | 0.0559 | 0.0864 | 0.3677 | 0.0545 | 0.3922 |
| Viridoalbina | 0.0672 | 0.0559 | 0.0326 | 0.2516 | 0.0251 | 0.0690 |
| Alboviridis | 0.0112 | 0.0419 | 0.0016 | - | 0.0011 | - |
| Viridoxantha | - | - | 0.0196 | - | 0.0033 | - |
| Xanthoviridis | - | - | 0.0016 | - | 0.0207 | - |
| Virescens | 0.1568 | 0.4748 | 0.2348 | 1.0644 | 0.1766 | 1.9875 |
| Chlorina | 0.1960 | 0.1396 | 0.2706 | 0.7744 | 0.1559 | 0.2311 |
| Lutescens | 0.0504 | 0.1257 | 0.0766 | 0.4258 | 0.0502 | 0.4160 |
| Albescens | 0.0784 | 0.1815 | 0.0310 | 0.3290 | 0.0131 | 0.3466 |
| Tigrina | 0.0896 | 0.0978 | 0.0929 | 0.3484 | 0.0480 | 0.2542 |
| Striata | 0.0560 | 0.0978 | 0.0619 | 0.2516 | 0.0055 | 0.0924 |
| Maculata | 0.2016 | 0.2793 | 0.2315 | 0.3677 | 0.1385 | 0.4853 |

*Agronomic trait mutations*

*Earliness*

Table III presents the frequency data for earlier maturing mutants selected at heading and maturity in the $M_2$ generation. The earlier maturing mutants were most frequent at the highest dose of each mutagenic treatment. Among them, the highest frequency was observed at 30 krad of gamma rays. Sodium azide produced the highest frequency of earlier maturing mutants at 0.004 M (0.0924%) but at the low dose (0.001 M) no earlier maturing mutants were detected despite the large population size. For gamma rays and EMS, the higher doses/concentrations promoted higher frequencies of early mutants.

*Plant height*

Short straw mutants were detected in the 20 krad gamma ray treatment and in the low EMS concentration (Table III). The gamma ray treatment produced the highest frequency, 0.0504%. Taller straw mutants were recovered with every mutagenic agent, with both doses of gamma rays and with the low doses of the chemical mutagens (Table III).

*Vigorous plants*

Vigorous and taller plants were selected at 30 krad gamma rays at a frequency of 0.1117%, and at the low dose of EMS at a frequency of 0.0032%.

TABLE III. FREQUENCY OF AGRONOMIC TRAIT MUTANTS IN THE $M_2$ GENERATION OF BUENAVISTA BARLEY, LA MOLINA, 1990-91.

| Mutagen treatment | Dose/ concent. | Earliness | | Height | | Plants (No.) |
|---|---|---|---|---|---|---|
| | | Heading | Maturity | Taller | Shorter | |
| Gamma rays | 20 krad | 0.0168 | 0.0112 | 0.0112 | 0.0504 | 17 855 |
| | 30 krad | 0.1257 | 0.0419 | - | 0.0279 | 7161 |
| EMS | 0.05 M | 0.0147 | 0.0016 | 0.0049 | 0.0082 | 61 341 |
| | 0.10 M | 0.0387 | 0.0193 | - | - | 5167 |
| SA $NaN_3$ | 0.001 M | 0.0109 | - | - | 0.0033 | 91 723 |
| | 0.004 M | 0.0231 | 0.0924 | - | - | 4327 |

*Other mutations*

Table IV presents the various morphological mutations induced by gamma rays, EMS, and sodium azide in the Buenavista variety of barley. Both doses of gamma rays induced more types of mutations than the chemical mutagens. The higher frequencies were found at both doses of gamma rays.

Rosette leaves were found in gamma rays, sodium azide, and the low dose EMS populations. The highest frequency, 0.1995%, was at the 30 krad dose. Narrow leaves were detected only in the 30 krad treatment population, 0.014%. Waxy plants were found at the high dose of the three mutagenic treatments, at a frequency that ranged from 0.014 to 0.0462%. Among the morphological mutants were two changes in the stem. One exhibited supernumerary nodes, 15-16, induced by the

high dose of gamma rays, and the other, plants with long internodes. The latter mutant type was recovered from populations of both gamma rays treatments and from the low concentrations of EMS. The frequency ranged from 0.0011% to 0.0231%.

Among the morphological mutations for head characters, irregular spikes were detected in all treatments, with the exception of the high concentration of EMS. The frequency varied from 0.0044% to 0.0952%, with the highest number in the gamma rays treated populations. Big lemma and palea occurred in the high dose populations of EMS and sodium azide at frequencies from 0.0194% to 0.0693%. Another modification of the spike was a wider awn base detected at the high concentration of EMS at a frequency of 0.0194%. Also observed was a waxy spike in the 20 krad gamma rays population at a frequency of 0.0224%.

TABLE IV. FREQUENCY OF MORPHOLOGICAL MUTATIONS OBSERVED IN THE $M_2$ GENERATION OF BUENAVISTA BARLEY TREATED WITH GAMMA RAYS, EMS, AND SODIUM AZIDE, LA MOLINA, 1990-91.

| Mutant traits | Gamma rays | | EMS | | Sodium azide | |
|---|---|---|---|---|---|---|
| | 20 krad | 30 krad | 0.05M | 0.01M | 0.001 M | 0.004 M |
| Rosette plant | 0.0056 | 0.1955 | - | 0.0194 | 0.0002 | 0.0693 |
| Narrow leaves | - | 0.0140 | - | - | - | - |
| Waxy plant | - | 0.0140 | - | 0.0194 | - | 0.0462 |
| Higher number of internodes | 0.0056 | 0.0016 | - | - | - | - |
| Long internodes | - | - | - | 0.0194 | 0.0011 | 0.0231 |
| Head | | | | | | |
| Six rows | 0.0336 | 0.0140 | 0.001 | - | 0.0055 | 0.0924 |
| Irregular rows | 0.0952 | 0.0559 | 0.003 | - | 0.0044 | 0.0231 |
| Compact type | 0.0168 | - | - | - | - | - |
| Lax type | - | 0.0140 | - | - | - | 0.0231 |
| Spikelet number | - | 0.0140 | - | - | - | 0.0231 |
| Awnless head | - | 0.0140 | 0.0016 | - | - | - |
| Short awns | - | - | - | 0.0194 | - | 0.0693 |
| Big lemma & palea | - | - | - | 0.0194 | - | 0.0693 |
| Wide awn base | - | - | - | 0.0194 | - | - |
| Waxy spike | 0.0224 | - | - | - | - | - |

*Naked kernel mutants*

Two methods of selecting naked kernels were applied: (a) Identification of mutants in the field at harvest. It was possible to identify mutant plants in the plots from the 30 krad gamma rays treatment, at a frequency of 0.02793; (b) Identification of mutant kernels in the laboratory in the harvested grain (Table V).

Sodium azide at 0.004 M gave the highest rate of naked kernels, although the differences among the treatments were not large. The $M_2$ mutants will be tested in the $M_3$ generation for confirmation.

TABLE V. FREQUENCY OF NAKED KERNELS IN TREATED POPULATIONS ($M_2$).

| Mutagen | Dose/ concentration | Naked kernels (No.) | Frequency (%) |
|---|---|---|---|
| Gamma rays | 20 krad | 781 | 0.091345 |
| | 30 krad | 1740 | 0.071166 |
| EMS | 0.05 M | 408 | 0.090666 |
| | 0.10 M | 3527 | 0.088065 |
| $NaN_3$ | 0.001 M | 543 | 0.120666 |
| | 0.004 M | 3451 | 0.070789 |

## 4. CONCLUSIONS

1. The mutation treatments conducted in 1986-89 were not effective in providing useful mutations primarily because of the uneven effect of the radiation unit.
2. In the 1990-91 period the application of radiation and chemical mutagens produced a large number of useful mutations.
3. The objective of inducing naked kernel mutants was achieved. Identifying this type of mutant was more effective in the laboratory than in the field.

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# IMPROVEMENT OF BARLEY AND *Chenopodium* SPECIES IN THE ANDEAN REGION BY INDUCED MUTATIONS

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**Abstract**

Experiments on the genetic improvement of barley (*H. vulgare*), quinoa (*Chenopodium quinoa*), and canahua (*C. pallidicaule*) were conducted in the field and greenhouse at the Centro de Investigaciones Nucleares at Viacha, using different concentrations and treatment durations of sodium azide ($NaN_3$) to induce beneficial mutations.

Greenhouse tests were conducted to establish the concentration and treatment duration for sodium azide. The treatments were applied and field evaluations were carried out in different generations to improve the germplasm available in the country, particularly in respect to environmental stresses such as frost, hail, and drought, which severely restrict yields.

## 1. INTRODUCTION

On the Bolivian Altiplano (high plateau), a series of factors influence and limit agriculture. The average rainfall is only 550 mm/year. The wettest months are December, January and February, and from April to October it is dry. The evapo-transpiration is higher than the rainfall. Frost, drought, and hail are frequent during the vegetative period of crops. The soils are deficient in both chemical and physical characteristics. All of these factors directly affect the yield of the different crops.

Barley is one of the leading traditional crops in Bolivia, being farmed for grain and mainly for fodder in the form of silage and hay for animal feeding. Barley tolerates drought and low temperatures better than wheat but it is affected by frost and drought in the reproductive stage during the spike formation period, resulting in either withered grains or no grains formed. Efforts to improve barley, therefore, are directed to developing varieties with a shorter vegetative phase, 80 to 100 days, to reduce the risk of drought or frost damage during the grain ripening period of the crop.

Quinoa is maintained for home consumption on the Altiplano and in the inter-Andean valleys. Around Titicaca Lake, farming is concentrated on the Copacabana peninsula, with a greater incidence in the Desaguadero area, and diminishing southward to Oruro. Quinoa has been used as food by the Andean population since ancient times because of the quality of the protein. It has a higher proportion of amino acids essential for human nutrition and a higher protein content than other cereals, being about 14 percent. The objectives of the mutation breeding programme in quinoa are to develop new varieties or lines with a larger grain size, lower saponine content and resistance to mildew (*Peronospora effusa*).

## 2. MATERIALS AND METHODS

After conducting the dosimetry tests with the mutagenic chemical agent (sodium azide) in the laboratory and greenhouse and optimizing the dose for barley ($10^{-3}$ M concentration for 2 hours), the treatment was applied to the seed of three barley varieties: IBTA 80, Criolla, and Kamiak. The seeds were sown in the field in the 1987-1988 agricultural year. The quantity of seed treated was 1 kg per variety. The seeds were sown at two sites, CIN-Viacha and CADEA-Oruro. The $M_1$ populations were

evaluated for seedling emergence, plant survival, and plant height.

The $M_2$ generation of the IBTA-80 and Criolla varieties was sown at CIN-Viacha in November of 1988. The populations were planted in rows spaced 0.35 m apart, with 15 cm between plants, covering an area of 540 $m^2$. Because of frequent frosts, only about 10% of the total population produced mature grains.

The $M_3$ generation was planted in November of 1989 with a 15 cm spacing between plants and 0.35 m between rows. The seedlings emerged uniformly 12 days after sowing. The tillering period was slow and extended into January, the period of highest rainfall. In the early reproductive stage the plants were damaged by low temperatures, so that no grain was formed and all of the material was lost.

In October of 1990, the $M_3$ was sown again, with reserve seed, at the same row and plant spacing as before. The germination was 80%. Tillering and flowering occurred 30 and 95 days after sowing, respectively. When grain ripening began, a hailstorm occurred, affecting 30% of the plants. Consequently, few spikes retained any grain, and the evaluations and selections that were planned could not be conducted. Only 120 spikes remained and were bulk harvested.

Quinoa seeds were irradiated with gamma rays ($^{60}Co$) at the IAEA Seibersdorf Laboratory, Austria, with a 30 krad (300 Gy) dose. Also, seeds of quinoa varieties Chucepace, Sajama, and Real-Royal were treated with three doses of sodium azide (0.5, 0.1, and 0.05%). The $M_1$ generation was grown at Condariri, near Oruro. The $M_2$ generation was sown at CIN-Viacha in October, 1989 on an area 360 $M^2$, in 50 rows, with a 0.5 m spacing between rows and 10 cm between plants. Germination was very poor, 10%. Only 176 plants emerged, with 141 reaching the panicle formation stage and 57 the mature grain stage. The 57 mature plants were harvested on an individual plant basis. In 1990, 57 $M_3$ lines were sown in 3-row plots. Germination of the different lines varied from 70% to 85%. The best panicles were harvested from each line that had a good size or diameter. The variety Royal was attacked by mildew, *Peronospora effusa*, which affected 80% of the plants, preventing panicle development and grain formation.

## 3. RESULTS AND DISCUSSION

In the dosimetry tests with barley, the most sensitive variety to sodium azide was Kamiak, with only 60% seedling emergence, compared with 75% and 67%, respectively, for IBTA-80 and Criolla (Table I). A number of plants of IBTA-80 and Criolla did not develop after emergence (Table II), and died. Kamiak never completed its vegetative cycle.

TABLE I. $M_1$ POPULATIONS DETAILS AND PLANT HEIGHT OF IBTA-80, CRIOLLA, AND KAMIAK CULTIVARS OF BARLEY.

| $M_1$ | IBTA-80 | Criolla | Kamiak |
|---|---|---|---|
| Seeds sown, No. | 1500 | 1500 | 1500 |
| Seedlings emerged, No. | 1125 | 1000 | 900 |
| Plants harvested, No. | 1095 | 965 | - |
| Mean plant height (cm) | 90 | 72 | 50 |

TABLE II. DURATION OF DEVELOPMENTAL STAGES IN THE $M_1$, $M_2$, AND $M_3$ GENERATIONS OF IBTA-80 AND THE $M_1$ AND $M_2$ GENERATIONS OF CRIOLLA BARLEY.

| Stage | $M_1$ | | $M_2$ | | $M_3$ |
|---|---|---|---|---|---|
| | IBTA-80 | Criolla | IBTA-80 | Criolla | IBTA-80 |
| Mean No. of days to: | | | | | |
| Emergence | 7 | 7 | 9 | 12 | 12 |
| Tillering | 35 | 42 | 49 | 51 | 58 |
| Booting | 60 | 75 | 65 | 72 | 72 |
| Flowering | 95 | 112 | 93 | 99 | 99 |
| Maturity | 130 | 137 | 145 | 145 | 145 |

All of the populations of quinoa derived from seed that had been irradiated at Seiborsdorf and planted at Viachai and Condoriri were lost due to frost. The $M_1$ generation of quinoa plants treated with sodium azide ranged in height from 40 to 70 cm, and panicle length ranged from 8 to 24 cm, with a 2 to 4 cm diameter.

The $M_2$ plants of quinoa (Table III) at Condariri had an average height of 60, 80, and 100 cm, respectively. Plants were harvested individually. During threshing, 811 plants with small grains were discarded. In the Sajame and Royal varieties, there was a greater variation in size and color than in the Chucepece variety.

TABLE III. DURATION OF DEVELOPMENTAL STAGES IN THE $M_2$ AND $M_3$ GENERATIONS OF QUINOA IRRADIATED WITH GAMMA-RAYS.

| Stage | $M_2$ | $M_3$ |
|---|---|---|
| Mean No. of days to: | | |
| Sprouting | 15 | 20 |
| Leaf appearance | 21 | 29 |
| Panicle formation | 60 | 67 |
| Flowering | 82 | 95 |
| Ripening | 152 | 185 |

At Condoriri, the $M_3$ generation (Table III) of the three varieties that had been treated with the three doses of sodium azide had an average development similar to that in the $M_2$ generation. Harvest was on an individual plant basis, saving a single tiller per plant. During threshing, plants with small grains were discarded. As in the previous generation, color variations were visible in the Sajama and Royal varieties, while the Chucepace variety was more stable.

# MUTATION BREEDING OF SORGHUM (*S. bicolor*) FOR TOLERANCE TO ALUMINUM CONCENTRATIONS

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**Abstract**

In Colombia sorghum is cultivated in different areas that have contrasting environmental conditions. This project was conducted in the Meta Department where there are problem soils; they contain high concentrations of aluminum and have a low pH. Also, the rainfall there is excessive. In the first stage of the project, seven cultivars were evaluated, of which two--Serere and IS-8577--were selected for the mutation induction programme.

The radiosensitivity test indicated that the optimal irradiation dose for Serere was between 300 and 350 Gy, and 300 Gy for IS-8577. Seven thousand seeds of each variety were irradiated with the selected doses. The $M_1$ generation was harvested on an individual panicle basis and the $M_2$ sown in panicle rows in cooperation with FEDEARROZ, a rice growers association, to conduct the selection for mutants.

## 1. INTRODUCTION

Sorghum cultivation in Colombia is facing several serious constraints that are limiting its expansion in terms of area occupied by the crop and the achievement of sustainable and stable yields. Therefore, the country is relying for its needs on costly imports from foreign markets.

The sorghum crop is used in cattle nutrition, with a production of ca. 673 000 tons on a cultivated area of ca. 255 000 ha in 1990. Because of various problems in the cultivation of Colombian varieties, 80% of the cultivated area in the early 1990's was in imported hybrids.

This project is being conducted in the Meta Department where the soils have a high aluminum content, low pH and low organic matter content. In general, they are sandy soils that are poor for farming. The high concentration of cattle in the area results in a high demand for feed concentrate, therefore the development of adapted varieties of sorghum is needed for wider cultivation and better exploitation of the soils in the region. The most suitable materials which have some degree of tolerance to aluminum and acceptable yield levels are the varieties Serere and IS-8577. Their cycle is 115 days, their plants are 1.80 m tall and their seeds are coffee brown.

## 2. MATERIALS AND METHODS

In 1989, seven cultivars of sorghum were evaluated in the field in order to establish the best parent varieties for mutation induction. The panicle length, disease resistance and aluminum tolerance were checked as parameters for selection. The best materials, Serere and IS-8577 (1051 line), were chosen, cleaned and stored in containers at a uniform temperature. These genotypes (Serere and IS-8577) were introduced through INTSORMIL from Kenya, their country of origin.

In the following year, uniform, plump, and dry seeds (14% moisture content) of the two cultivars were irradiated with 0, 100, 200, 300, 400 and 500 Gy. For the preliminary test, fifty seeds per replication per dose were irradiated with $^{60}Co$ gamma source (Canada C188 model) at a dose rate of 3000 R/minute at Instituto de Asuntos Nucleares (IAN), Santafé de Bogotá (Colombia), to determine the radiosensitivity curve. After treatment, the seeds were sown in beds in the greenhouse. The height of 20 day-old seedlings was recorded and doses for the mutagenic treatments were chosen: 300 and 350 Gy for Serere; and 300 Gy for IS-8577.

Seven thousand seeds of each variety were included in each dose and the control, and were sown on April 8, 1990, at Espinal. Although the $M_1$ was grown in isolated plots, cross pollination control was performed in part of the population, using bags. The $M_1$ generation plants were harvested individually and sown in the field in the following year as panicle rows. The mutants were to be selected on the basis of the main objectives of the research: tolerance to high aluminum content, height of plants, earliness and yield performance.

## 3. RESULTS AND DISCUSSION

The radiation treatments reduced the germination rate and seedling height with increasing gamma ray doses. Similar results were published by IAEA (1977) and Prina (1989) in respect to the correlation between seedling height, germination and gamma ray doses in the $M_1$ generation. There were significant differences in germination when the dose and varieties were considered. There were significant differences in seedling height between the different doses. There were no significant differences in height between varieties.

In this experiment, seedling height was used as a parameter of the somatic effect of the radiation doses. Growth reduction of 50% for Serere was caused by 470 Gy and for IS-8577, by 450 Gy. The following treatment doses were chosen: 300 and 350 Gy for Serere, with a growth reduction of 25% and 35%, respectively; and 300 Gy for IS-8577, with a growth reduction of 35%. In 1991, seven thousand seeds per treatment per variety were irradiated and individual $M_1$ plants were harvested as follows: Serere--1650 plants, 300 Gy; 1870 plants, 350 Gy; 875 plants, 0 Gy; IS-8577--1845 plants, 300 Gy; 727 plants, 0 Gy. The $M_2$ seeds were prepared for sowing in the field in the following year for selection, in cooperation with Fedearroz (Rice Growers' Association).

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# CONCLUSIONS AND RECOMMENDATIONS

## GENERAL

The conclusions and recommendations reflect the focus of the programme. They deal mainly with cereals but a few deal with other crops. The conclusions and recommendations are arranged by crops and by approaches.

Under this CRP, mutant lines were developed with disease resistance, earliness, semi-dwarfness, tolerance to aluminum and excellent yield performance relative to the respective controls, indicate that through induced mutations it is possible to select mutants for direct release as varieties for the farmers. However, induced mutations should be considered as complementary to conventional breeding programmes, because most mutants serve as sources of desirable genes. Segregating populations derived from crosses can form the foundation of conventional breeding programmes and will continue to be important.

The following general approaches were recommended:

- Use well adapted varieties and look for the traits desired such as dwarfness, earliness and disease resistance.
- Relatively low doses of radiation should be used to induce desirable changes and recover useful mutants.
- In comparing the effectiveness of three selection methods: pedigree, modified pedigree and modified bulk, the greatest number of mutant lines was obtained with the pedigree method (in wheat).
- In evaluating the performance of semidwarf mutants the layout of the yield trials must be adapted to the architecture of semidwarfs and full consideration given to population density, row spacing, soil fertility, border effects and sprinkler irrigation when feasible.
- The selected mutants should be evaluated in different areas to determine their yield potential and stability

## RICE

Induced mutations were an effective breeding tool in achieving specific objectives that involve readily identifiable qualitative recessive traits. Mutant lines were identified that combined the desirable characteristics of the parent variety such as vigor, plant type, lodging resistance, and threshing resistance, with fine, translucent grains. Some were also very early, resistant to "hoja blanca", and had higher resistance to *Sogatodes*.

In the evaluation of the $M_2$ and $M_3$ generations, it was concluded that selection for morphological characters in the $M_2$ generation was effective and the mutants were confirmed in the $M_3$ generation. Early maturity and grain shape are two of the easiest mutations to detect by simple observations or measurements; mutations for these characters can be easily induced.

Irradiation of rice seeds with gamma rays increased genetic variability and produced a number of mutants in respect to the growth cycle, plant height, lodging resistance, chlorophyll deficiencies, maturity, various morphological traits, reaction to iron toxicity, grain quality factors, plant architecture, disease reaction, and grain yielding ability.

The spectrum and yield of mutations varied between varieties. Gamma rays and neutrons gave different mutation spectra in the same varieties for a wide range of characters studied. The possibility of obtaining various combinations of desirable characters is a very important reason for utilizing ionizing radiations of different LETs and chemical mutagens in a mutation breeding program.

It is important to utilize effective screening techniques for blast reaction to assure that disease-free plants are truly resistant, rather than susceptible plants that escaped infection. Considering the fact that the parental varieties are highly susceptible to blast, caused by *Pyricularia grisea*, it was concluded that a significant level of resistance was developed in the selected mutants.

A combined seed treatment, 2.5 x $10^{-3}$ M $NaN_3$ + 25 Gy, caused the highest percentage of chlorophyll mutants in the greenhouse. In the field it induced mutants for earliness (one even 20 days earlier), a different hull color, and shorter plants classified as dwarf, semidwarf and intermediate. In the greenhouse, the 200-250 Gy dose range decreased germination 40% and seedling height 60%. In the field, the reduction in surviving plants was 70% and in seedling height 70%. In the single and combined treatments (gamma rays and sodium azide), the treatments 1.0 x$10^{-3}$ M $NaN_3$ + 25 Gy and 2.5 x $10^{-3}$ M $NaN_3$ +25 Gy reduced the height of the seedlings 29-37% and the surviving plants 28-51%. The treatment 2.5 x $10^{-3}$ M $NaN_3$ + 25 Gy produced more chlorophyll mutants than 1.0 x $10^{-3}$ M $NaN_3$ + 25 Gy. The same treatment also resulted in mutants that were shorter and earlier than the parent variety.

Anther culture

Anther culture of *indica* rice is very difficult. The regeneration of green plants is usually below 1%. There are genotypic variations with regard to the capacity of their pollen to produce calli, although the calli production rates are very low.

In some cases, but not always, gamma irradiation of seeds enhanced callus induction from $M_1$ plants but there were differences between hybrids. As the radiation dose increased, callus induction decreased. However, the radiation increased the capacity of the calli to regenerate green plants. The efficiency of green plant production from anthers was highest in the 100 Gy treatment, 0.97 green plants/100 plated anthers. The second highest was the 200 Gy treatment, with 0.49%. Lowest was 400 Gy with 0.15%. The control (0 Gy) was not efficient in green plant production, with 0.22%.

**WHEAT**

Through the use of radiation there was an increase in genetic variability, making possible the selection of several genotypes: semidwarf plant height, an increase in resistance to diseases and higher yields. Earliness appeared to be very beneficial under certain environmental conditions, since several lines with this trait were superior to the controls in yield, 1000 grain weight, hectoliter weight and leaf-rust resistance. It is possible that the early mutant lines were more productive because they escaped diseases that reduced the yields of later maturing genotypes.

In certain wheat genotypes the mutant lines showed higher variability for grain yield than for plant height in relation to the original cultivar. Also, a tendency for greater height reduction among the lower yielding lines was observed.

The results with some wheat mutants indicate that adaptability and stability are not correlated. In practical terms, selection for greater adaptability (geographic stability) does not guarantee stability under different climatic fluctuations across years (temporal stability). It is possible to obtain genetic improvement for temporal stability in wheat, but it would be more difficult to accomplish than for yield.

*In vitro* culture

A higher percentage of callus development was achieved in anthers incubated in the dark, as compared to those kept constantly under light conditions. There was a wide range of genotypic variation for callus induction capacity. Also, there were genotypic differences in the proportion of albinos among the regenerated plantlets.

External sterilization with ethanol produced a higher number of sterile anthers as compared to the NaOCl treatment. Nevertheless, callus induction was clearly higher when chlorox was used instead of ethanol. This might be attributable to a higher dehydration of anthers by the use of ethanol. Cold pre-treatment and dark incubation showed favourable effects on androgenesis, while application of osmotic pressure had no effect. Anther extraction should be done when the apex of the spike reaches 3/4 of the distance between the base of the sheath of the second leaf and the ligule of the flag leaf.

In some genotypes, 2,4-D at 2 mg/l plus kinetin at 1.5 mg/l were the two growth regulators that increased the androgenic response best. Solid Potato-4 medium increased callus formation and plant regeneration as compared to the liquid form; however, the opposite was true for $N_6$ medium, where the liquid form resulted in increased plant regeneration and fewer albino plantlets. This same effect was observed when the $N_6$ medium was used together with Ficoll-400. Sucrose concentration affected callus induction. Plant regeneration was obtained with 9% sucrose. The cold treatment applied to anthers before culturing affected plant regeneration, but not callus induction. Untreated anthers, or anthers treated for two days, gave the higher number of regenerated plants. Anthers derived from plants which developed from seeds treated with 250 Gy had a lower response to callus induction compared with anthers derived from non-treated seeds or treated with 50 or 150 Gy.

In certain wheat varieties the best combinations of media and hormones on immature embryo culture were MS, Potato II, and $N_6$ basal media, each supplemented with 1 mg/l 2,4-D, 2 mg/l 2,4-D, and 2 mg/l 2,4-D plus 0.5 mg/l kinetin. All gave 100% callus induction. Plantlets were not obtained in the plant regeneration phase, but when calli from the best callus induction treatments were transferred to MS basal medium, they produced a good number of green points.

## TRITICALE × WHEAT

The great variability in the populations from the irradiated $F_1$ seeds from triticale x wheat crosses provides the programme with many possibilities for selection. From the irradiated triticale × wheat crosses it was possible to find improved lines for hectoliter weight associated with significant improvement in yield.

## BARLEY

Wide variability was observed in the chemical mutagenic treatments, numerous chlorophyll mutations were recorded. The mutant types included albina, xantha, alboxantha, xanthoalba, viridoalbina, viridoxantha, xanthoviridis, virescens, chlorina, lutescens, albescens, tigrina, striata and maculata. Earliness and changes in the spike morphology were observed in most cases. The earlier maturing mutants were most frequent at the highest dose of each mutagenic treatment. Short straw mutants were detected in some mutagenic treatments as well as tall straw mutants. Other morphological mutations induced by gamma rays, EMS, and sodium azide in barley were rosette leaves, narrow leaves, waxy, supernumerary nodes and long internodes. The identification and selection of naked kernals was more effective in the laboratory, after the harvest, than in the field during the harvest.

## SORGHUM

Doses of 300-350 Gy were established for treatments of sorghum seeds of certain genotypes with gamma rays.

## BEANS

Hypocotyl explants are good sources for callus induction. Callus induction was 100% when the hypocotyl explants were cultured in MS basal medium supplemented with 5 mg/l NAA + 0.5 mg/l kinetin. The heaviest callus fresh weight was produced by the hypocotyl and embryo. When root explants were cultured in $B_5$ basal medium supplemented with 0.1 mg/l 2,4-D + 0.5 mg/l kinetin, or 0.5 mg/l 2,4-D + 0.5 mg/l kinetin, callus induction was 100%.

## POTATO

High efficiency of X, S, and Y virus elimination by thermotherapy was established. Difficulty was experienced in eliminating X and Y viruses by meristem culture. The best response in tuber production was induced by adding 10 mg/l BAP to the basal medium. Also, the largest tuber weight was obtained when 10 mg/l BAP were added to the basal medium.

## QUINOA

Preliminary mutagenic studies were conducted in quinoa (*Chenopodium quinoa*) with gamma rays and sodium azide. These studies should be enhanced.

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