THE APPLICATION OF NUCLEAR ENERGY TO AGRICULTURE

Annual Report

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Prepared by

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CONTENTS

PERSONNEL iv

GRADUATE ASSISTANTS v

RESEARCH

A. MUTATION BREEDING AND RADIATION BOTANY

1. Mutation Induction in Manihot 1

a. Somatic mutations 1

b. Mutations induced by pollen irradiation 3

c. Persistance of the R_1 mutants induced by pollen irradiation 7

d. Advantages of pollen irradiation 7

e. Possible use of the induced mutants in Manihot breeding 8

2. A Compact Mutant in Coffee 10

3. Field Trials and Nutrition Study of the White Bean Mutant 13

a. Yield trials 16

b. Nutrition study 17

4. Radiosensitivity of Tropical Plant Species 20

B. CONTROL OF INSECTS BY MALE STERILIZATION METHOD

1. Sterilization of the Mediterranean Fruit Fly and Its Application to Fly Eradication 23

a. Further studies on mating competitiveness of medfly males irradiated during pupal or adult stage 23

b. Induction of visible medfly mutants by gamma radiation 30

2. Studies on the Biology and Sterilization of the Coffee Leaf Miner, Leucoptera coffeella (Guerin-Meneville) 31

a. Competitiveness of gamma sterilized males of the coffee leaf miner, irradiated as adults 31

b. Inherited sterility among the progeny of male coffee leaf miner treated with gamma irradiation 35

c. Mating competitiveness of F_1 males 38

3. Biology and Sterilization of the Shootborer *Hypsipyla grandella* Zeller (Lepidoptera: Phycitidae) 42

C. RADIOBIOLOGY IN INSECT PATHOLOGY

1. Pathological Control of Insect Pests 43

a. Susceptibility of *Hypsipyla grandella* Zeller to *Bacillus thuringiensis* var. *thuringiensis* and *Bacillus thuringiensis* var. *entomocidus* 43

b. Growth of *Hypsipyla grandella* Zeller reared on a synthetic diet 46

c. Determination of the LD_{50} of *Metarrhizium anisopliae* on fifth instar larvae of *Hypsipyla grandella* Zeller 55

d. Susceptibility of *Dermatobia hominis* to *Bacillus thuringiensis thuringiensis* 66

2. Radiation Biology and Mutagenesis of Insect Pathogens 71

a. Survival of *Metarrhizium anisopliae* spores after ultraviolet or gamma rays irradiation 71

b. Comparative survival of several varieties of *Bacillus thuringiensis* after ultraviolet irradiation 77

D. SOIL CHEMISTRY

1. Exchange Properties of Volcanic Soils 79

a. Properties of clay minerals from volcanic ash soils 79

	b. CEC determination by cation exchange resin	89
2.	Phosphate Chemistry In Tropical Soils	95
	a. Quantity/Intensity Parameter	95
	b. E-value in soils of Central America	98
3.	Trace Elements in Tropical Soils: Zinc adsorption in volcanic ash soils	101

A. MUTATION BREEDING AND RADIATION BOTANY
(C. C. Moh and J. J. Alan)

1. Mutation Induction in Manihot

a. Somatic mutations

In the past year's Annual Report, we have described the botanical characteristics and the agricultural significance of this basic root crop in the lowland tropics. Since Manihot is usually propagated by vegetative means, we are investigating whether or not induction of somatic mutations is an effective means for the improvement of this crop. The radiosensitivity of the shoot apexes of stem cuttings, which are normally used as propagules, has been studied. The results showed that an acute dose of 4 kr of gamma radiation was about the maximum that the shoot apexes could tolerate. A dose above this level (e.g. 5 kr) critically inhibited their growth. By using a dose of 4 kr of gamma radiation to irradiate the stem cuttings, we explored the somatic mutation rate in seven cultivars in our Manihot collection. The mutation frequencies are presented in Table 1.

It can be seen from the irradiation results that most of the cultivars produced very low frequencies of somatic mutations even though a sub-lethal dose of gamma radiation of 4 kr was given. Only cultivar No. 82 had a higher mutation rate. Further irradiation experiment confirmed this result (Table 2). The higher mutation rate of cultivar No. 82 is probably due to a higher degree of heterozygosity in

Table 1. Somatic mutation rate of seven _Manihot_ cultivars induced by irradiating the stem cuttings

Cultivar identification number	Gamma radiation dose (kr)	No. of cuttings irradiated	No. of shoots emerged	Mutations observed in R_1	
				No.	%
9	0	25	66	0	0
	4	50	48	3	6.2
16	0	25	61	0	0
	4	50	24	0	0
23	0	25	56	0	0
	4	50	15	1	6.7
26	0	25	64	0	0
	4	50	15	0	0
68	0	25	66	0	0
	4	50	41	1	2.4
71	0	25	64	0	0
	4	50	30	1	3.3
82	0	25	67	0	0
	4	50	48	6	12.5

Table 2. Somatic mutation rates induced by gamma radiation in _Manihot_ cultivar No. 82

Dose (kr)	No. of cuttings irradiated	No. of shoots emerged	Somatic mutation	
			No.	%
0 (ck)	40	106	0	0
2	40	78	3	3.9
3	40	71	4	5.6
4	40	57	11	19.3

this cultivar.

Many mutations produced from irradiated cuttings ap-peared in chimeric forms. In order to obtain a homogeneous plant of the mutated character, it is necessary to grow the mutated cutting for some generations to purify the mutated sector. However, we have obtained a number of homogeneous mutated plants in the second vegetatively propagated generation by growing the chimeric cuttings.

The types of somatic mutations induced can be classified into two groups: chlorophyll and morphological. The most common types of chlorophyll mutations were yellow, orange-yellow, pale green and light green. Most of these mutations came from cultivar No. 82. The morphological mutations included notched leaf, curly leaf, narrow lobe and miniature. Almost all these morphologically mutated characters had an effect on growth which usually showed a stunted or semi-dwarf effect.

b. Mutations induced by pollen irradiation

Since the somatic mutation rate induced by irradiating stem cuttings was rather low, we were searching for other methods of inducing mutations in Manihot. Obviously, seed irradiation is not an effective means, because of the chimera production and the protogynous flowering habit which prevents self-fertilization to bring out the recessive mutations. Subsequently, we used pollen irradiation method which showed promising results.

In our Manihot collection, there are 90 cultivars which came from Central and South America. Each cultivar and its origin was identified by a cultivar number. The cultivars used for the present investigation were No. 56 and No. 68 in the collection. Number 56 has a good root quality with yellow root core, but the growth habit is prostrate and is male sterile. Number 68 has a high root production, an erect growth habit, and no floral sterility. The roots of both cultivars take about 8 to 10 months for harvesting.

Mature pollen were collected from cultivar No. 68 at anthesis and were irradiated with an acute radiation dose of 2 and 3 kr from a cobalt-60 source. The pistillate flowers of cultivar No. 56 were protected with a bag made of nylon mosquito netting several days before the flowers opened. This process prevented the possible out-crossing made by insects. The irradiated pollen were immediately pollinated to the pistillate flowers which were then again protected with the nylon bag until the fruits matured. This process was necessary and advantageous because it allowed us to collect the seeds when the fruits dehisced explosively to eject the seeds at maturity.

The hybrid seeds from irradiated pollen as well as non-irradiated pollen (serving as a control) were stored in a dry area for 4 to 8 weeks and were grown individually in small Fertil Pots in the greenhouse. The germination of the seeds was generally low. We found that less than 30% of the seeds from irradiated as well as non-irradiated pollen could germinate and develop into seedlings. When the seedlings were 1 to 2 months old, they were transplanted to the nursery for further development.

All the mutations reported here were isolated in the R_1 generation. A mutant consistently expressing its mutated character for more than 6 months of growth was recorded as a mutation.

The mutation frequencies obtained from pollen irradiation with 2 radiation doses are presented in Table 3. While the radiation doses used for the present experiment were not high, the frequencies of mutation obtained were appreciable. Thus pollen irradiation appears to be an effective method for inducing mutations in Manihot. It must be pointed out that all the mutations reported here were obtained in the R_1 generation. The frequencies of recessive mutations in the R_2 generation for these 2 radiation doses have not been explored, because of the difficulty of self-fertilization which arises from the protogynous flowering habit in Manihot. To plant the stem cuttings from an R_1 plant at different time intervals, however, would give a possibility of synchronizing the opening periods of the staminate and pistillate flowers, and would permit self-fertilization to bring out the recessive mutations. This study is being carried out in our laboratory.

Most induced mutations found in the R_1 generation were

Table 3. Frequency of mutations in the R_1 generations induced by pollen irradiation in Manihot, Cultivar No. 56 x cultivar No. 68

Radiation dose (kr)	No. of R_1 plants	Mutations[1]	
		No.	%
0 (control)	72	0	0
2	45	8	17.8
3	55	21	32.8

[1] Most mutations observed were due to morphological changes which included the changes in leaf size (small or narrow leaf-lobe), leaf shape (wrinkle or curly leaf), the plant form (dwarf or miniature), and growth vigor. Chlorophyll mutations were very few, and were limited to yellow mottling and yellow-green:

morphological mutations. Of the 29 R_1 mutants obtained from the present irradiation experiment with the 2 radiation doses, 24 (83%) were classified as morphological mutants and only 5 (17%) were chlorophyll mutants. These results give a striking difference from those obtained from seed irradiation in many plant species in which most of the induced mutations obtained in the R_2 generation are of a chlorophyll-deficient nature.

The morphological mutations induced by pollen irradiation varied considerably from mutant to mutant. In fact, we found no two mutants were exactly alike. Most commonly, however, they were changes in leaf size, leaf shape, plant form, and growth vigor. Not many types of chlorophyll mutation were induced; so far they have been limited to yellow mottling and yellow-green.

c. Persistence of the R_1 mutants induced by pollen irradiation

All the induced mutant characters reported here appeared to be a permanent change rather than a temporary physiological disturbance. Stem cuttings from the R_1 plants were propagated in the field and those with the mutant characters in the R_1 also expressed the identical characters in the subsequent vegetatively propagated generation. Since all the mutations were produced in the R_1 and no mutations (which could arise from the crosses of the two cultivars due to their heterozygous conditions) were found in the control population, it can be considered that the induced mutations are of a dominant nature. However, dominant true gene mutations are extremely rare. It is likely that many of the induced mutations were the results of chromosomal aberrations.

d. Advantages of pollen irradiation

The obvious advantages of pollen irradiation method for Manihot breeding is to eliminate the chimera production. The R_1 plants can be grown and immediately evaluated for their agricultural value. Desirable mutants, once recognized and vegetatively propagated, can then be tested and released as improved cultivars. This greatly facilitates the breeding process without carrying out the work on purification of the chimeric sector.

Another advantage of pollen irradiation is that when a pollen is irradiated, only a haploid set of the chromosomes is affected by the radiation. The other set of chromosomes of the egg nucleus is not irradiated and thus is unaffected. Therefore, the resulting zygote, after fertilization, would have less genetic or chromosomal damage and have a better chance of survival to reveal the mutational changes. This may explain our present results that a rather high frequency of mutations in the R_1 generation can be induced with a relatively low dose of gamma radiation. Manihots are allopolyploids (Jenning, 1963). Polyploids usually have a higher ability to tolerate chromosomal aberrations than do diploids (Nilan, 1956. Sparrow, et al, 1961) which may also be attributed to the phenomenon that a high mutation rate was induced by pollen irradiation in Manihot.

e. Possible use of the induced mutants in Manihot breeding

As has been pointed out previously, there was a wide variation in plant morphology and growth vigor among the R_1 progenies from pollen irradiation. This large variation gave us an excellent opportunity for making selections of the desirable types. Indeed, we have isolated a number of mutants with vigorous growth (Figure 1) for our breeding program. We are searching for the early mutants which produce mature roots in less than 8 months for the semi-arid areas of the Pacific coast of Central America. If the vigorous mutants produce sizeable edible roots in 6 to 7 months, they can be considered as early mutants. These mutants are

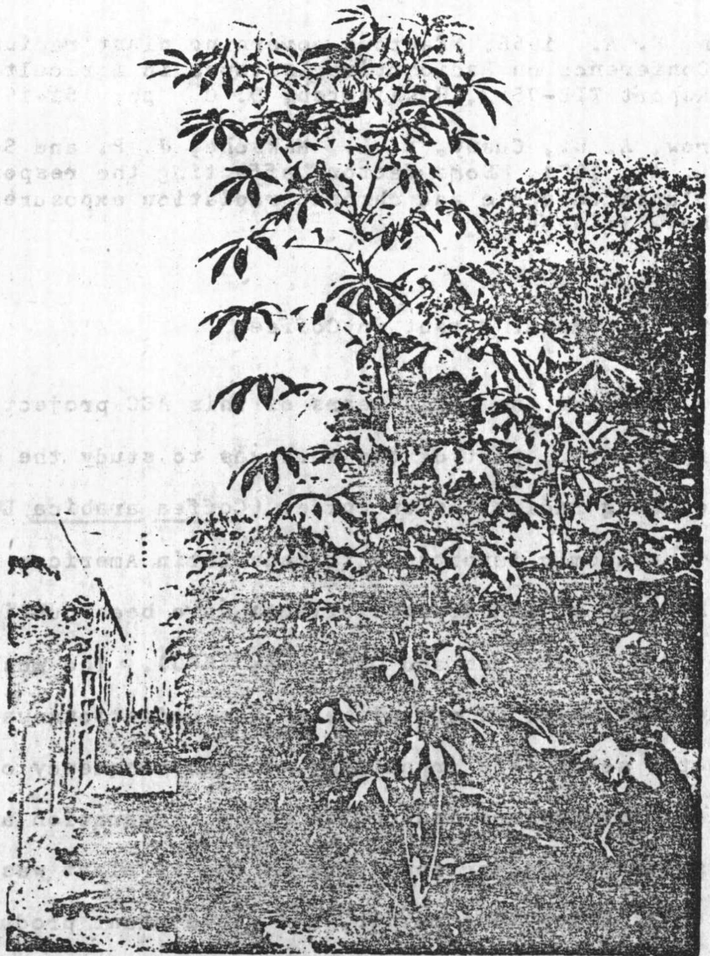

Fig. 1. A vigorous mutant of Manihot among the R_1 progenies induced by pollen irradiation.

being vegetatively propagated for field trials.

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2. A Compact Mutant in Coffee

During the early years of this AEC project at Turrialba Center, part of the work was to study the effects of ionizing radiations on coffee (Coffea arabica L.) because of its economic importance to many Latin American countries. The biological and genetic effects have been published earlier (Moh, 1962; Moh and Obregoso, 1960). The most unusual biological response of coffee to the radiations was that when the seeds were irradiated, a high frequency of morphological mutants was induced in the R_1 generation and that the mutant character appearing in the R_1 plant was not a sectorial change but rather, the whole plant produced a similar mutant character (Moh, 1961). This evidence suggests that the development of a young coffee shoot is from a single initial cell of the corpus.

In an experiment designed for inducing mutations for breeding purposes, samples of coffee seeds (*Coffea arabica* var. *typica*), 100 seeds per sample, were irradiated with a series of radiation doses from 2.5 to 20 kr from the ^{60}Co source in the gamma field (in 1958, the only radiation source then available in the project). The irradiated seeds were grown in the nursery and the R_1 plants were then transplanted to the field for further observation.

In 1961, the R_1 plants began to reach a full grown stage and to produce fruits. One plant in the R_1 population irradiated with 5 kr showed many desirable characteristics and has drawn much of our attention. This plant was semi-dwarf, had short internodes and compact floral clusters, and produced fruits rather heavily. Seeds of this plant were then propagated and the progenies showed a segregation from the parental type to the very dwarf type. Evidently, the semi-dwarf compact R_1 mutant was due to incomplete dominance.

Since the characteristics of this semi-dwarf compact mutant are very desirable, effort has been made to isolate a homozygous line for agricultural practice. After four generations of selfing and selection, we have obtained homozygous lines of this semi-dwarf compact type of plants. The average plant height was about 6 feet which greatly facilitated the fruit harvesting. The internodes were short, and the fruit clusters grew compactly along the fruiting branches. Figures 2 to 5 show the morphology of the semi-dwarf compact mutant.