IAEA-TECDOC-260

INDUCED MUTATIONS FOR IMPROVEMENT OF GRAIN LEGUME PRODUCTION II

PROCEEDINGS OF THE
SECOND RESEARCH CO-ORDINATION MEETING
ON THE USE OF INDUCED MUTATIONS FOR IMPROVEMENT
OF GRAIN LEGUME PRODUCTION IN SOUTH EAST ASIA
ORGANIZED BY THE
JOINT FAO/IAEA DIVISION
OF ISOTOPE AND RADIATION APPLICATIONS OF ATOMIC ENERGY
FOR FOOD AND AGRICULTURAL DEVELOPMENT
HELD AT CHIANG MAI, THAILAND
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FOREWORD

Following recommendations of a Regional Seminar in Sri Lanka 1975 (IAEA-TECDOC-234), a co-ordinated research programme on the Use of Induced Mutations for the Improvement of Grain Legume Production in South East Asia was established stepwise in 1976-77. In 1978 it became part of the IAEA Regional Co-operative Agreement (RCA) with Member Countries in South East Asia. A first research co-ordination meeting was organized, 28 May - 1 May 1979 at Kuala Lumpur (Malaysia), at which discussions centred around methodology of mutagen treatments and management of the treated populations. Among the objectives, special attention was given to disease resistance (IAEA-TECDOC-234).

This document contains full papers or extended summaries of research progress reports as well as the conclusions and recommendations of the second research co-ordination meeting two years later. The meeting was organized, 27 April - 1 May at Chiang Mai (Thailand) with generous assistance from the Department of Agriculture, Bangkok, which is gratefully acknowledged. Besides discussing the status of ongoing projects, particular attention was given to the matter of symbiotic nitrogen fixation. The subject was introduced by a special lecture from Dr. C. Atkins, Botany Department, University of Western Australia at Nedlands and has been considered under the aspect of at least maintaining present levels of N_2 fixation during breeding programmes for higher grain production but also with a view towards improvements of the symbiosis.

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RESEARCH PROJECT REPORTS

SPECIAL LECTURE

INDUCED MUTATIONS FOR NEW PLANT TYPES AND DISEASE RESISTANCE IN MUNGBEAN AND BLACKGRAM*

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ABSTRACT

Mungbean and blackgram are important grain legumes in Bangladesh, however, the productivity is low. Varietal improvement should be a basic requirement for increasing production. Following treatment of seeds with ^{60}Co gamma rays a number of positive deviants were selected having improved yield factors.

INTRODUCTION

Mungbean (Vigna radiata (L.) Wilczek) and blackgram (Vigna mungo) acreage in Bangladesh are ca. 15,607 and 52,297 hectares with annual productions of ca.9,540 and 38,350 tons, respectively. Average yields of the two crops are 622 and 745 Kg/ha (Anonymous, 1976-77). The cultivars grown are old and are obviously low-yielding types. From the breeding points of view, the low productivity of the cultivars may be attributed to :

1. Undesirable plant types such as plants with less number of branches, pods and seeds, spreading and bushy habits and trailing tendency under abundant moisture regimes.
2. Asynchronous habit of pod maturity resulting in decreased seed yield/plant (Ahmed et al. 1978).
3. Yield reduction due to infection with diseases like Yellow Mosaic Virus, Cercospora leaf spot and Powdery Mildew.

Since there is not much scope for making a significant breeding advance due to the absence of a broad genetic base in these crops (Shaikh et al. 1980a), a mutation breeding programme was initiated for creating variability in the existing cultivars and selecting desirable lines.

MATERIALS AND METHODS

Dry seeds (10-12% moisture) of two accessions of mungbean, MB-55 and MB-56, and two of blackgram, B-10 and B-23, were irradiated with 50-90 kR doses of ^{60}Co gamma-rays. These accessions were developed at INA through single plant selections from unnamed local cultivars. In all 4000 and 3000 seeds of mungbean and blackgram, respectively were

* Supported by IAEA under Research Contract No. 1921/RB.

irradiated and the M_1 generation was grown keeping the accessions isolated from each other by 100 m each way. A total of 1723 M_1 plants of mungbean and 1382 of blackgram were chosen for collecting M_2 seeds. The first-formed two pods of each M_1 plant were harvested and bulked dose-wise. A similar set was prepared from the control plots.

In the M_2 generation (1979-80), all the seeds thus collected were sown in the field keeping 30 and 15 cm distances between rows and seeds, respectively. Usual cultural practices were carried out during the growing period. The easily identifiable variants i.e. "macro-mutants" were selected out from the M_2 populations.

During the M_3 generation (1980-81), seeds of the "Macro-mutants" were sown in plant-progeny rows for identifying true-breeding lines. One row of each of the respective mother variety was sown after every five plant-progeny rows for ensuring effective comparisons. Spacings of 30 and 15 cm between rows and plants, respectively were used. Agronomic data on number of primary branches, inflorescences and pods per plant, plant height, pod length, number of seeds/pod, seed size (500-seed weight) and seed yield/plant were collected from ten plants of each progeny-line and the respective mother variety. Detailed comparisons, both visual and on the basis of data, were made between the M_3 populations and those of the respective mother variety and true breeding lines were selected out on the basis of these comparisons.

RESULTS AND DISCUSSION

Mungbean

The types and frequency of "macro-mutants" obtained in the M_2 population of mungbean are presented in Table 1. Total number of variants observed among the M_2 progeny of accessions MB-55 and MB-56 were 108 and 110, respectively. The variant types were synchronous, early maturing, broad- and narrow-leaved, bushy, erect, dwarf, deep green, large and small podded, black podded and upright podded. When the two strains are considered together it is observed that maximum variants were synchronous in pod maturity, followed by narrow-leaved, dwarf, early maturing and erect plant types. It is also clear that higher percentages of variants were obtained in the M_2 populations following higher doses of irradiation.

In the M_3 generation, only 9 and 10 true-breeding lines were isolated from plant-progeny rows of 108 and 110 M_2 plants of the accessions MB-55 and MB-56, respectively (Tables 2 & 3). The salient features of these mutants were: Dwarf, erect, shiny and large podded, bold seeded, broad-leaved, synchronous pod maturity, tall and bushy types. The mean agronomic data of these mutants (Tables 2 & 3) reveal a lot of variations among the mutants and the respective mother varieties. Remarkable

improvements in a few characters of some mutants were noticed. These were: number of primary branches in MB-55(4), MB-55(9), MB-55(10), MB-55(13), MB-55(18), MB-56(54), MB-56(63) and MB-56(95); number of pods in all mutants of MB-55 and MB-56 except one; number of seeds/pod in MB-55(6), MB-55(16), MB-55(17), MB-56(54), MB-56(102) and MB-56(69). It is also interesting to note that some mutants of both the accessions produced higher seed yield/plant than the respective mother varieties.

Blackgram

The various types of "macro-mutants" isolated in the M_2 populations of blackgram, their frequency and mean of agronomic characters are shown in Table 4. There were 217 and 150 variants among the M_2 populations of the accessions B-10 and B-23, respectively. It is evident that the types of variants included deformed leaves, early and late maturity, and, erect, bushy and trailing plants. In general, maximum percentage of variants were observed in 60-90 kR doses. The most frequent type of variability, considering the two strains together, was deformed leaf followed by erect, trailing, bushy and early maturing types.

From among the M_3 plant-progeny rows of B-10 and B-23, seven and 10 true-breeding lines were identified, respectively (Tables 5 and 6). The salient features of these lines were: early, erect, synchronous, broad-leaved, upright pod, dwarf, bushy, spreading, bold podded, resistant to diseases like Yellow mosaic virus, Powdery mildew and Cercospora leaf spot. The variations observed among the mutants and the respective mother varieties in respect of the agronomic characters are shown in Tables 5 and 6. Improvement of some characters like number of pods/plant in mutants B-10(23), B-10(36), B-10(59), B-23(26), B-23(81), B-23(90), seed size in B-10(23), B-10(59) and B-23(90), number of primary branches in B-23(92) and resistance to diseases in B-10(25) and B-10(63) are remarkable.

The first-formed two pods instead of all the pods of the M_1 plants were collected considering the economy in mutation experiments (Redei, 1974 and Brock, 1979) and also with the idea that M_2 seeds thus collected would presumably contain more mutants than those from later-formed pods since natural elimination of radiation-induced aberrations had been found to occur with progressive growth of the M_1 plants of two other grain legumes (Shaikh and Godward, 1974).

The variant types which were selected in the M_2 generation but did not breed true in the M_3 were physiologically disturbed (Mia et al. 1966). It also became clear from the data that treatment with higher doses of gamma-rays used in these experiments yielded higher frequency of mutants in both the crops.

The remarkable improvement in characters like numbers of primary branches and pods/plant and seeds/pod in mungbean and synchrony of pod maturity and disease resistance in blackgram are encouraging. Number of pods and primary branches have been found to be positively and significantly correlated with seed yield/plant in mungbean (Ahmed et al. 1981). partial and multiple correlation studies also revealed the highest influence of pod number on seed yield. However, some of these characters and the higher seed yield/plant were not reproduced exactly in the M_3 generation. This is expected because these are quantitative characters and are under polygenic controls. But the characters like synchrony of pod maturity and disease resistance were more stable and might have resulted from "point-mutations". Some mutants with similar nature were obtained through gamma-irradiation in mungbean (Ahmed et al. 1978; Haq and Shakoor, 1980; Shakoor and Haq, 1980)and other grain legumes such as Lathyrus sativus (Shaikh, 1972), Vicia ervilia (Shaikh and Godward, 1975), Cicer arietinum (Shaikh et al. 1978). One Cicer mutant, M-669, with increased number of pods/plant, 20% higher seed yield and 4% more protein content has been named Hyprosola (H=high; Y=yielding; PRO=high protein; SOLA=Bengali for chickpea) (Shaikh et al. 1980b). It is expected that it will be possible to either release some of these mutants directly as varieties or use in hybridization programmes for improving these crops.

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Table 1. Types and frequency of mungbean variants observed in the M_2 generation (1979-80).

Strains	Doses kR	Total M_2 plants studied	Synchronous maturity	Early maturity	Leaf type		Plant type			Large podded	Bold seeded	Other types		Total No. variants	% variants observed
					Broad	Narrow	Bushy	Erect	Dwarf						
MB - 55	0	1500	-	-	-	-	-	-	-	-	-	-		-	-
	50	1408	5	7	6	10	4	-	-	-	1	Deep green	1	34	2.4
	60	432	5	10	1	7	2	-	1	1	2	upright pod	1	30	6.9
	70	331	3	-	1	3	2	1	4	2	1	-		17	5.1
	80	203	2	2	-	4	2	1	1	2	-	-		14	6.9
	90	168	-	-	-	5	2	2	2	2	-	-		13	7.7
Total			15	19	8	29	12	4	8	7	4	2		108	
MB - 56	0	1000	-	-	-	-	-	-	-	-	-	-		-	-
	50	690	8	6	1	1	4	1	7	4	1	-		33	4.8
	60	636	8	3	1	-	1	2	7	1	-	Black pod	1	24	3.8
	70	642	7	-	1	-	-	4	6	1	-	-		19	3.0
	80	464	4	1	-	6	2	8	1	2	-	-		24	5.0
	90	119	-	-	-	-	-	2	2	5	-	Small pod	1	10	8.4
Total			27	10	3	7	7	17	23	13	1	2		110	

Table 2. True-breeding M_3 lines from the mungbean accession No. MB-55, their identifying characters and agronomic data (1980 - 81).

Accession/ Mutant No.	Selected from gamma-ray dose (kR)	Plant height (cm)	No. of primary branches/ plant	No. of inflore-scences/ plant	No. of ineffective pods/ plant *	No. of effective pods/ plant *	Pod length (cm)	No. of seeds/ pod	500-seed weight (gm)	Seed yield/ plant (gm)	Identifying character(s)
MB - 55	Control	36.1	2.1	6.7	2.4	35.5	5.8	10.3	3.2	6.2	
MB - 55(6)	50	40.0	2.1	8.0	4.3	29.9	6.2	14.2	7.5	6.2	Synchronous
MB - 55(16)	60	29.5	3.5	22.2	36.5	40.6	6.3	14.3	6.2	7.5	Large podded
MB - 55(17)	60	37.3	2.2	10.1	19.8	30.5	6.0	13.5	8.6	7.1	Bold podded
MB - 55(18)	60	26.4	4.1	28.2	48.5	47.7	6.1	13.1	6.1	7.2	Bushy & synchronous
MB - 55(10)	70	41.8	6.0	21.3	60.2	54.3	5.8	12.5	5.9	8.1	Erect, Many podded
MB - 55(13)	70	29.2	5.3	13.1	13.1	52.9	5.6	8.4	7.5	6.5	Shiny black pod colour
MB - 55 (4)	80	37.1	5.2	23.5	43.5	56.6	6.5	11.3	6.4	7.8	Synchronous
MB - 55(9)	80	35.1	5.1	30.2	37.5	61.8	6.1	10.2	5.7	7.4	Erect, synchronous
MB - 55(2)	90	26.0	4.1	19.1	21.1	67.3	5.0	7.1	7.5	6.8	Bushy

* "Effective pods" means pods having at least one healthy seed and "ineffective pods" included unfilled, immature and insect-damaged pods.

Table 3. True-breeding M_3 lines from the mungbean accession No. MB-56, their identifying characters and agronomic data (1980 - 81).

Accession/ Mutant No.	Selected from gamma-ray dose (kR)	Plant height (cm)	No. of primary branches/ plant	No. of inflore-scencos/ plant	No. of ineffective pods/ plant *	No. of effective pods/ plant *	Pod length (cm)	No. of seeds/ pod	500-seed weight (gm)	Seed yield/ plant (gm)	Identifying character(s)
MB - 56	Control	29.7	1.4	6.6	2.4	29.2	5.3	7.5	11.5	5.5	
MB - 56(54)	50	14.2	3.0	12.8	5.7	27.8	7.5	12.4	9.0	7.5	Dwarf, large podded
MB - 56(55)	50	22.3	2.5	11.4	1.5	39.0	5.3	6.2	10.4	5.6	Shiny pod
MB - 56(56)	50	28.4	2.2	11.5	2.6	35.8	5.4	6.2	10.8	5.9	Erect plant
MB - 56(102)	50	37.0	1.2	8.4	1.0	22.3	5.6	12.1	11.9	7.8	Large pod, bold seed & erect plant type
MB - 56(63)	60	24.5	3.5	11.3	4.2	30.0	6.2	8.3	10.2	5.3	Broad leafed
MB - 56(67)	60	18.3	2.2	10.2	0	25.8	5.8	8.5	11.6	6.2	Synchronous in pod maturity
MB - 56(69)	60	21.1	2.4	10.1	0	19.1	7.1	12.2	12.0	7.1	Synchronous in pod maturity
MB - 56(76)	70	19.5	1.2	7.2	1.2	18.0	6.5	10.3	11.5	5.3	Dwarf & erect
MB - 56(94)	80	35.3	1.3	8.5	1.5	15.0	6.3	11.1	11.5	5.5	Tall & erect
MB - 56(95)	80	30.4	4.5	13.0	3.4	26.2	5.8	10.5	10.2	6.4	Bushy type

* "Effective pods" means pods having at least one healthy seed and "ineffective pods" included unfilled, immature and insect-damaged pods.

Table 4. Types and frequency of blackgram variants observed in the M_2 generation (1979-80).

Strains	Doses kR	Total plants studied	Deformed leaf	No.of sterile/ partially sterile plants	Maturity		Plant type			Total no. variants	% variants observed
					Early	Late	Erect	Bushy	Trailing		
B - 10	Control	600	-	-	-	-	-	-	-	-	-
	50	532	27	4	-	-	6	5	6	48	9.0
	60	455	36	1	7	2	4	5	7	62	13.6
	70	268	10	3	7	3	-	5	6	34	12.7
	80	240	13	1	6	4	4	5	4	37	15.4
	90	220	9	-	8	6	3	7	3	36	16.4
Total			95	9	28	15	17	27	26	217	
B - 23	Control	400	-	-	-	-	-	-	-	-	-
	50	340	14	1	2	5	6	6	4	38	11.2
	60	279	12	1	1	4	6	6	2	32	11.5
	70	263	13	3	-	4	9	2	6	37	14.1
	80	229	5	6	7	3	6	2	5	28	10.6
	90	121	4	3	3	2	2	-	1	15	12.4
Total			48	14	13	18	29	16	18	150	

Table 5. True breeding M_3 lines from the blackgram accession No. B-10, their identifying characters and agronomic data (1980 - 81).

Accession/ Mutant No.	Selected from gamma-ray dose (kR)	Plant height (cm)	No. of primary branches/ plant	No. of inflore-scences/ plant	No. of ineffective pods/ plant *	No. of effective pods/ plant *	Pod length (cm)	No. of seeds/ pod	500 seed weight (gm)	Seed yield/ plant (gm)	Identifying character(s)
B - 10	Control	24.8	2.5	12.3	2.0	18.5	4.2	6.2	16.7	3.8	
B - 10(20)	60	11.9	2.7	9.8	1.1	19.0	4.3	6.6	16.4	4.1	Dwarf, early
B - 10(23)	50	22.1	2.9	12.7	11.6	18.2	4.8	6.5	23.4	5.6	Erect, synchronous, upright pod
B - 10(25)	50	15.6	2.0	11.8	3.2	22.0	4.5	6.3	18.0	5.0	Dwarf, dark-green leaf, resistant to YMV, powdery mildew and Cercospora leaf spot
B - 10(36)	40	18.4	3.2	14.6	2.2	25.3	4.7	6.5	14.6	4.9	Semidwarf, sparsely hairy
B - 10(58)	30	15.8	2.7	12.0	2.1	21.6	4.8	6.8	17.8	5.1	Bushy, spreading, Semi-dwarf
B - 10(59)	30	21.4	2.8	13.3	9.2	20.3	4.8	6.7	23.3	5.8	Bold podded
B - 10(63)	20	16.8	3.6	9.3	1.2	22.1	4.3	6.7	18.4	5.1	Semi-dwarf, resistant to powdery mildew

* "Effective pods" means pods having at least one healthy seed and "ineffective pods" included unfilled, immature pod insect-damaged pods.

Table 6. True breeding M_3 lines from the blackgram acc.No. B-23, their identifying characters and agronomic data (1980-81)

Accession/ Mutant No.	Selected from gamma-ray dose (kR)	Plant height (cm)	No. of primary branches/ plant	No. of inflore-scences/ plant	No. of ineffective pods/ plant *	No. of effective pods/ plant *	Pod length (cm)	No. of seeds/ pod	500 seed weight (gm)	Seed yield/ plant (gm)	Identifying character(s)
B - 23	Control	20.9	3.2	10.7	2.3	20.0	4.1	6.4	16.7	4.2	
B - 23(22)	90	15.2	2.0	4.0	2.0	17.0	4.5	6.0	16.2	4.5	Early, synchronous
B - 23(26)	80	30.0	4.0	24.0	33.7	25.3	4.5	6.8	16.2	4.7	Synchronous
B - 23(35)	70	15.5	2.0	6.0	0	18.0	4.3	6.0	16.3	4.6	Erect, synchronous
B - 23(78)	60	15.6	2.0	8.0	0	16.0	4.9	6.5	17.0	5.4	Early and erect
B - 23(79)	60	24.2	3.0	13.0	8.4	30.6	4.5	6.5	16.4	4.9	Early
B - 23(81)	60	29.0	3.0	23.0	17.5	28.5	4.0	6.8	17.2	5.5	Broad leafed
B - 23(82)	50	28.7	3.0	13.0	9.6	27.4	4.1	6.4	16.5	4.9	Erect, synchronous
B - 23(90)	50	21.6	3.0	20.0	28.2	28.8	4.2	7.2	20.1	6.0	Bushy
B - 23(92)	50	23.5	4.0	18.0	12.0	25.0	4.0	6.8	19.5	5.8	Synchronous
B - 23(16)	50	23.4	3.0	12.0	3.7	25.3	4.9	7.0	18.5	5.7	Erect

*"Effective pods" means pods having at least one healthy seed and "ineffective pods" included unfilled, immature and insect-damaged pods.

A MUTATION BREEDING PROGRAMME FOR IMPROVING SOME GRAIN LEGUME CROPS IN EGYPT[1]

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ABSTRACT

A mutation induction experiment using gamma rays and several chemical mutagens aims at mutants of Phaseolus vulgaris and Vicia faba with improved yield characteristics and resistance to pests. A number of promising mutants have been selected and are being examined in advanced generations.

INTRODUCTION

Beans (Phaseolus vulgaris) and field beans (Vicia faba), are of the major legume crops grown in Egypt. Each of these crops suffers from some handicaps; such as low yielding ability, susceptibility to pathogenic microorganisms and other parasites, unfavourable plant type ... etc. Due to the narrow genetic variability among the cultivated varieties, conventional breeding methods did not contribute much to their improvement. Therefore, we thought that induced mutations may be an effective tool to generate new variability, which can be utilized to overcome some of the handicaps facing these crops. A few years ago, we initiated a mutation breeding programme, using gamma-rays, EMS, NaN_3 (sodium azide) and combinations of these mutagens. The objectives are to select mutants with desirable characters for direct use or for use in cross-breeding.

I. Phaseolus vulgaris

1.1 Objectives:

(a) Selection of some useful mutants with altered ontogenetic pattern, such as a changed flowering time, higher yielding ability, and improved protein quality and quantity.

(b) Test of mutants for resistance to some pathogenic microorganisma; such as rust (Uromyces phaseoli) and viral diseases.

(c) Test of selected mutants for response to the local strains of Rhizobium.

1.2 Material and Methods:

The bean variety "Contender" was used as a starting material. It is widely grown for green use in Egypt. It flowers after about 5 - 6 weeks from sowing. It has moderate yield; and seeds are buff with brown mottling. Its protein content ranges between 22 to 23 %. It is susceptible to rust and mosaic.

Size of Experiment:. About 9,000 seeds.

1.3 Results:

All M_1-plants that showed good fertility (based on number of seeds/plants) and normal growth habit were harvested individually. The progenies

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(1) IAEA-RC-No. 2466

of each M_1-plant were grown as single families in M_2 generation. The M_2-population size reached about 7,000 plants. Selection in M_2 mutagenized populations was practiced for the following traits; a) Flowering time (early and late) (b) Plant type (c) Higher yield

In general, selection intensity in M_2 was about 8 %. A summary of M_2-population size and mutants selected is given in Table 1.

1.3.1 Behaviour of selections in M_3 and M_4 generations:

Progenies of the M_2-selections were grown in M_3 each as a separate M_3-family. The results of M_3 generation can be summarized in the following points:

(a) Some of the late or early flowering mutants selected in M_2 proved to be true deviants from the parent variety. However, many M_3-families restored the flowering time level of the control. These families had been discarded from further test.

(b) All indeterminate plant type mutants bred true.

(c) The majority of the leaf-shape modifications, selected as mutants in M_2, did not breed truely in M_3 generation. Therefore, they were excluded from further test.

(d) Some higher yielding mutants retained their potentialities during M_3.

Selection on individual plant basis was practiced in M_3 generation within the families that showed deviation from the control. Progenies of each M_3- selection were grown again as families in M_4. The results for flowering time mutants in M_4 are given in Table 2. The results for higher yield mutants in M_4 are presented in Table 3.

1.3.2 Behaviour of flowering time mutants in M_5 Generation:

Seeds of all plants of each flowering time mutant (family) in M_4 were harvested and bulked together. In general variation within each M_4 family was, more or less equal to variation for flowering time of the control material. Each M_4-family was considered as a mutant. The bulked seeds of each family were used to grow the mutants, in a larger scale, during M_5. A replicated trial was carried out. The results of flowering time mutants in M_5 generation are presented in Table 4.

1.3.3 The glabrous and indeterminate mutants:

Data concerning different traits of two glabrous and five indeterminate Phaseolus mutants, selected during this study are presented in Table 5.

1.3.4 "Giza-80" A new mutant variety of Ph. vulgaris:

From a previous mutation breeding programme, initiated in 1973, (Hussein and Disouki, 1976 and 1979), a white seed coat colour mutant, showing some improved attributes was selected, and maintained through selfing for more than 10 generations uptill now. This mutant was obtained after gamma irradiation (10 Kr) of air-dry seeds of a French variety called "Fin de Villeneuve" imported, several years ago for green use in Egypt. Table 6 summarizes the different attributes of the mutant and its parent.

Electrophoresis analysis for the seed proteins of the mutant and its parent showed clear cut differences in the banding patterns of the two genotypes.

At present, mutant variety "Giza-80" is under test for response to nodulation with regard to the local strains of Rhizobium phaseolii. It is also under test for productivity, under different climatic conditions in some private farms.

II. Vicia faba

Most of the field bean varieties grown in Egypt belong to the equina-type. Unfortunately, none of these varieties seem to be resistant to Orobanche. They suffer from shortage in genetic variability in this respect. Therefore, we thought that induced mutations may be a useful tool to enrich this variability. We started out mutation breeding programme in November 1979, with the objectives of selecting mutants resistant to Orobanche; either for direct use, or for use in cross-breeding.

II.1 Material and Methods:

Seed samples of the local variety "Giza-2" were treated as follows:

γ-rays : 10 and 20 Kr
EMS : 0.05 %, 0.10 % and 0.15 %; at pH = 7
NaN_3 : 1×10^{-3} M, 2×10^{-3} M, and 3×10^{-3} M; at pH = 3.5
NMU : 0.5×10^{-3}, 0.75×10^{-3} and 1×10^{-3} M.

We have chosen these doses or concentrations according to our experience from previous work. Treatment conditions are mainly those described by Hussein & Abdalla (1974) and by Brunner (1977). The M_1-size, including control, was more than 6,000 seeds.

II.2 Results: (a) M_1-Generation:

(1) The germination of control was more than 80 %.

(2) The germination of the irradiated material ranged between about 60 - 70 %.

(3) Depending on the concentrations the germination of the material treated with chemical mutagens ranged between 40 - 50 %.

(4) As a general observation, all M_1-surviving plants resulting from the NMU -treatment were vigorous, but unfortunately they showed almost complete sterility. Therefore, they were dropped from this experiment.

(5) At harvest time, all M_1-plants from each mutagenic treatment were collected together and their seeds were bulked.

(6) M_2-Generation: In November 1980, the M_2 generation was sown in the field. Before sowing, the seeds were mixed with Orobanche seeds. Moreover, the experimental field itself was highly contaminated with Orobanche seeds from previous seasons. (Note that the seeds of the parasite plant can remain dormant in the soil for more than 16 years). In addition, seed samples of the different M_2-populations were mixed with seeds of the parasite plant and sown in pots. The soil in the pots was artificially made contaminated with Orobanche seeds.

The M_2-population size of these experiments, was more than 5,000 plants that survived until flowering.

Preliminary results of M_2:

(1) All M_2-mutagenized material showed segregation for chlorophyll mutants. This is considered as a good criterion for the efficiency of the mutagenic treatments applied.

(2) In the field experiment, severe attack with the parasite plant started to appear during the flowering period, and continued during fruiting. However, many plants, whether within the control material, or within the mutagenized M_2-population were parasite-free. The situation was less severe in the pot experiment.

As a general observation, many of the infected plants with the parasite plant did not develop pods. Those showed delay in infection

developed less number but smaller pods, and probably smaller and less number of seeds. On the contrary, non-infected plants fruited normally. As the plants are still, at present in the field not fully ripe, I cannot give data for their yielding ability. We do not know, if any of these normally growing plants is truely resistant or not. Our criteria of selection will be as follows:

(1) Selection for host plants of higher seed weight. This is based on the idea of Cubéro (1973) in Spain "that minor genes for host resistance are associated with polygenes for host seed weight". Natural selection favours plants with more seeds.

(2) Selection for host plants with less number of Orobanche stems (Boorsma, 1980).

Table 1. M_2 - population size and survey of mutants selected (selection intensity ≃ 8 %)

Treatment in M_1	No. M_2.pl. screened	No. of selections in M_2						
		late*	early*	indet.pl. type	higher yield	leaf shape	Total	%
γ-rays	939	54	20	2	18	4	98	10.4
EMS	1730	70	31	3	35	21	160	9.3
NaN_3	1293	33	44	4	51	16 + 2**	150	11.6
γ-rays + EMS	623	17	12	-	7	2	38	6.1
γ-rays + NaN_3	679	28	26	3	14	3	74	10.9
EMS + NaN_3	695	17	10	-	4	1	32	4.6
Control	958	-	-	-	-	-	-	-
Total	6918	219	143	12	129	49	452	$\bar{x}$ = 7.6

* Fl.t. of control variety 40 days.
** Glabrous mutants.

Table 2. Performance of fl. time mutants relative to control in M_4 - generation.

Mutant	fl.t. in M_3 days	size in M_4	Mean (days) in M_4	Behaviour in M_4	Mutagen
Control	38 Med. late	73	34.2	Medium late	-
R-1-L	56 late	42	39.6	late	γ-rays
R-2-L	56 late	22	38.7	late	
R-3-L	56 late	21	40.7	late	
E-1-L	55 late	25	36.0	late	EMS
E-2-E	21 early	23	30.1	early	
E-3-E	22 early	23	31.7	early	
N-1-L	55 late	42	37.0	late	NaN_3
N-2-L	55 late	39	39.3	late	
N-3-E	20 early	35	31.0	early	
N-4-E	20 early	28	32.2	early	
N-5-L	55 late	31	40.1	late	
N-6-L	55 late	34	41.2	late	
N-7-E	20 early	30	30.1	early	
N-8-E	20 early	31	29.2	early	
EN-1-E	20 early	27	27.4	early	EMS + NaN_3
EN-2-E	21 early	36	28.6	early	
RE-1-E	21 early	31	29.5	early	γ-rays + EMS
RE-2-L	55 late	40	30.1	early	
RN-1-L	55 late	27	43.4	late	γ-rays + NaN_3
RN-2-L	56 late	27	44.6	late	
RN-3-L	55 late	23	38.7	late	

Table 3. Performance of higher yield mutants in M_4 generation.

Mutant	No. of plants scored	Mean + S.E. (g/plant)	Mutagen
Control	50	20.6 ± 1.2	-
R-1-HY	38	25.4 ± 1.2	γ-rays
R-2-HY	31	24.7 ± 1.4	
R-3-HY	38	25.2 ± 1.2	
N-1-HY	40	26.9 ± 1.4	NaN_3
N-2-HY	44	25.6 ± 0.9	
N-3-HY	31	27.1 ± 1.3	
N-4-HY	34	24.4 ± 0.9	
N-5-HY	40	26.3 ± 1.2	
N-6-HY	36	26.2 ± 1.1	
E-1-HY	48	25.9 ± 1.1	EMS
E-2-HY	39	24.8 ± 1.3	
E-3-HY	36	26.5 ± 1.3	
E-4-HY	31	27.2 ± 1.6	
RE-1-HY	44	26.5 ± 1.3	γ-rays + EMS
RE-2-HY	38	26.2 ± 1.4	
RN-1-HY	42	28.4 ± 1.4	γ-rays + NaN_3
RN-2-HY	40	26.5 ± 1.3	
RN-3-HY	39	25.8 ± 1.2	
EN-1-HY	38	27.4 ± 1.1	EMS + NaN_3
EN-2-HY	44	27.8 ± 1.4	

Table 4. Performance of flowering time mutants in M_5 generation.

Mutant	Behaviour in M_4	No. pl. in M_5	Mean in M_5 (days)	Behaviour in M_5
Control	Med. late	316	35.5	Med. late
R-1-L	late	310	36.5	late
R-2-L	late	211	40.2	late +
R-3-L	late	305	42.7	late +
E-1-L	late	214	38.5	late +
E-2-E	early	304	30.4	early +
E-3-E	early	216	29.1	early +
N-1-L	late	201	40.5	late +
N-2-L	late	314	41.8	late +
N-3-E	early	251	34.2	early
N-4-E	early	331	34.3	early
N-5-L	late	316	40.6	late +
N-6-L	late	304	43.7	late +
N-7-E	early	295	36.5	late
N-8-E	early	211	34.8	early
EN-1-E	early	304	27.2	early +
EN-2-E	early	219	30.1	early +
RE-1-E	early	253	27.4	early +
RE-2-E	early	270	29.2	early +
RN-1-L	late	311	41.5	late +
RN-2-L	late	281	43.7	late +
RN-3-L	late	219	36.1	late

+ will be selected for further test.

Table 5. Performance of different traits of glabrous and indeterminate mutants during M_4 generation (50 plants/item).

Mutant	Fl. t. (days)	No. Pods/ pl.	Seed wt. (g/pl.)	No. Seeds/ (pl.)	Protein (%)
Gl. 1	37.6	14.7	20.8	46.2	22.6
Gl. 2	36.4	14.2	23.5	67.1	22.7
Control	35.4	13.6	21.7	48.4	22.1
In - 1	39.6	11.2	22.5	55.4	21.4
In - 2	36.4	12.0	24.7	48.5	22.6
In - 3	38.5	12.4	23.4	56.4	21.3
In - 4	37.1	14.2	21.2	56.2	22.5
In - 5	37.0	13.2	21.6	44.6	21.4

Gl = glabrous In = indeterminate

Table 6. Comparison between mut. Var. "Giza-80" and its parent Variety "Fin de Villeneuve"

Mutant "Giza-80"	Percent Find, Vill.
White seed Coat	Mottled violet seed coat
Rust resistant	Rust susceptible
About 12 % higher yield than	Fin d. Vill.(Mean 4 years)
Bigger seed size	Smaller seed size
(100 seed wt = 33 g)	(100 seed wt $\simeq$ 24 g)
For green and dry use	for green use only
Cooking time for dry	about 30 - 35 min.
seed about 13 - 15 min.	
straight green pods	straight pods with violet dots
Protein % $\simeq$ 24 %	Prot. % $\simeq$ 22 %

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MUTATIONAL IMPROVEMENT OF PIGEON PEA (*Cajanus cajan* L. Millsp.) FOR PLANT ARCHITECTURE AND GRAIN YIELD

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Abstract

Pigeon pea is an important grain legume in India, grown mainly under marginal and high risk conditions but with poor yield. Improved productivity is expected from changing the plant architecture via mutation induction. An experiment was started using gamma rays. A wide variability of characters affecting grain yield was created. Selected mutants are under evaluation.

Introduction

Pigeon pea is a tropical grain legume. It is grown under a wide range of conditions, on poor soils without supplemental nitrogen, under scanty rainfall conditions without irrigation, and as monocrop or intercrop, predominantly with cereals like sorghum, maize and millets. In India it occupies 2.5 million hectares yielding 1.8 million tonnes of grain and is the second important grain legume. Under the high risk, low management and subsistence semi-arid farming systems pigeon pea is a widely preferred grain legume intercrop component due to its hardiness and slow early growth. Cultivars with 100 to 300 days maturity duration are grown. Pigeon pea is a woody perennial retaining some of the wild habits and characters. The plant grows in large vegetative mass under favourable conditions, produces pods in flushes, sheds leaves under moisture-stress conditions and enters another cycle of vegetative and reproductive phases with moisture availability. Forms with synchronous flowering and annual-like habit are also available. Only very little work had gone in to the improvement of this important legume. Under the subsistence farming conditions pests, mainly pod borer (Heliothis armigera) and pod fly (Melanagromyza obtusa) take a heavy toll of the crop. Present yield level which is very low could marginally be improved and stabilized through better crop and pest management. Increasing productivity to make the crop suitable for commercial cultivation is the matter of primary importance in pigeon pea breeding. A real breakthrough in productivity of this crop, however, is possible only if the plant is restructured both morphologically and physiologically to increase the harvest index (Swaminathan, 1972). Mutation breeding has the recognized potential to bring about novel changes in plant structure and productivity.

MATERIAL AND METHODS

Dry seed of a medium maturing variety,Hy-2(150 days duration),was irradiated with 15,20,25,30,35,and 40 kR in a gamma cell available at the Division of Genetics,Indian Agricultural Research Institute,New Delhi.Control and treated seeds were sown directly in the field with 2 feet x 1 foot spacing.Germination and survival of seedling were recorded 7 and 21 days,respectively after sowing.Observations were made for morphological variants at maturity.All pods of individual plants were harvested separately.

Raising M_2 Generation: M_2 generation was sown as plant-to-row progeny.Wherever sufficient seeds were available 40 seeds were sown in a row of 20 feet.Germination data were collected 7 days after sowing and at two leaved stage seedlings were screened for chlorophyll deficient mutations.One month after germination seedlings were thinned out to one foot intra-row spacing,retaining a maximum of 20 plants in a row.Observations for viable mutations affecting leaf shape,plant type,flowering duration and floral and pod characters were taken during and after flowering.Observations on quantitative characters like plant height,number of primary and secondary branches were recorded on five randomly selected plants from each progeny row.Flowering date,grain yield,number of seeds per 10 pods and 100-seed weight were recorded on the entire M_2 populations.Progeny from 15 kR treatment was dropped due to the low mutation frequency.Single plants which were superior in yield, branching,number of seeds per pod,distinct in plant type and with earliness were selected.

Raising M_3 Generation: A total of 860 M_2 single plant selections and similar selections from control were advanced to M_3 generation. All available seeds of early maturing mutants were sown in a seperate block along with control.High yield selections were advanced in three rows having a maximum number of 60 plants in each family.Three rows of control were sown with every 60 rows of selections.Selections with increased flowering branches and seeds per pod were sown in two rows,maintaining a maximum plant stand of 40 per family.Two rows of control were sown with every 60 rows of selections.Flowering date and uniformity of flowering were recorded in all families and control.Five plants chosen at random from each family were observed for number of primary and secondary branches,seeds per 10 pods,grain yield,total plant weight and 100-seed weight.Single plants selected for plant type and yield were seperately harvested.Yield of all M_3 families was also recorded.

RESULTS AND DISCUSSION

Results on M_1 generation germination, survival and morphological variants are summarised in Table 1. Germination was low in all gamma ray treatments and the irradiation effect was drastic at doses above 25 kR. Exposure at 35 and 40 kR had considerable effect on seedling growth and vigour. Less vigourous plants flowered late.

Table 2 presents results on M_2 generation germination, and frequency of chlorophyll deficient mutations. Chlorophyll deficient mutations comprised of albina, xantha and variegata. Frequency of chlorophyll mutations expressed as percentage of M_2 seedlings showed a linear relationship with increase in radiation dose. Decline in the observed frequency of mutations recovered from the 30 and 35 kR treated progenies appears to be due to the low survival of their M_1 plants. Results in general tend to suggest that optimum mutation frequency and plant survival could be achieved at 25 kR treatment.

M_2 generation variability for flowering duration is presented in Table 3. Irradiation, in general, did not shift the mean flowering duration. But it did create variability for the character. Eight early flowering plants were identified from the 25 kR treated population. One of them is a month earlier to the mother variety. Early flowering habit bred true in M_3 generation. Grain yield of these mutants in M_3 was 12 to 48% less than the control (Table 4). In pigeonpea grain yield is positively associated with maturity duration. Some of these early mutants, however, had an improved harvest index. Earliness in these mutants apparently had brought in a rapid dry matter accumulation rate and increased productivity per day.

Single plant yield in M_2 generation presented in Table 5 indicates the wide variability observed. Uniformity of yield among control population was very low. Increased variability was, however, noted in irradiated populations with the occurrence of few high yielding plants. Mean yield in all treated populations were lower than that of control. Yield in pigeonpea is mainly contributed by number of pods, number of seeds per pod and 100-seed weight. Number of pods is generally dependent on the number of flowering branches. Number of seeds per pod is a varietal character with high heritability (Pandey, 1972) and appeared to yield limited variability on irradiation (Table 6). Hundred seed weight, on the other hand, is highly heritable (Sharma, et al., 1972) and amenable to radiation induced variability. M_2 variability for 100-seed weight ranged from 8.7 to 16.6g, while in control it was 10.8 to 13.7g with a mean of 12.1g. Increased seed weight appeared to adversely affect the number of developed pods. However, with larger variability available for these traits it is possible to improve them simultaneously. One way of increasing

number of pods is by selecting for higher flowering termini.Results presented in Tables 7 and 8 indicate that induced variability is possible for higher flowering termini.

239 high yielding M_2 selections were advanced to M_3 generation.Mean M_3 family yield in all families was much lower than the respective M_2single plant yield.However,in some M_3families few single plants outyielded their parental M_2 plants.This wide fluctuation in yield between M_2 and M_3 generations is not unexpected because grain yield in pigeonpea has low heritability(Munoz and Abrams,1971;Khan and Rachie,1972).Therefore rigorous progeny testing of early generation selections under different environments would be most effective in identifying true high yielding genotypes.High yielding single plants were also isolated from some of the 357 M_3 families derived from M_2 selections for increased seeds per pod. 162 M_2 plants selected for increased flowering termini did not show uniformity and consistency for the trait in M_3 generation. It appeared that number of flowerings termini in pigeon pea is not a highly heritable character.

Variation in plant type was found to be very difficult to adequately describe in quantitative terms.Mother variety,Hy-2 has tall semi-spreading habit,4-8 pods are formed on a peduncle which is spatially distributed along the branch.Higher number of primary branches with lesser secondary branches (Table 7 and 8) renders the plant a semi-spreading appearance.An optimum number of primary branches with more secondary branches ,on the other hand, gives a compact appearance and provides more flowering and pod formation sites.Plant compactness may hence be expressed as an index of total termini to number of primary branches,which is being termed as termini index.Plant types with high termini index and flowering termini have potential for increased yield per plant and permit more plants per unit area.Higher M_2 variability was observed for secondary branches than for primary branches(Tables 7 and 8). Altered plant types isolated in M_2 included compact plants with increased flowering termini and termini index.Flowering pattern was also found altered in some compact plant types.Internodal length of flowering termini in these plants was highly condensed and the flowers on the branch appeared in clusters.High grain yield was not always observed with altered plant types.However,high yielding M_2 and M_3 single plant selections had increased flowering termini and number of pods.

Restructuring of pigeonpea plant type in addition to alteration in morphological frame must involve favourable physiological change for efficient transformation of inputs in to grain.It is not clear what alteration in which morphological structure could confer physiological efficiency in grain legumes.

Therefore, from breeders point of view increased yield and harvest index are useful selection parameters in assessing physiological efficiency of new plant types.Harvest index in control and M_2 population showed considerable variation.There was very low uniformity for harvest index in control and it ranged from 7.5 to 22.0 with a mean of 17.4.In irradiated population the variation ranged from 8.2 to 27.4.High harvest index in M_2 selections when advanced to M_3 generation showed no consistency for the character like the grain yield.Frequency of plants with high yield and harvest index was lower in M_3 than in M_2 generation.Some of these selections had altered plant type.Harvest indices of 860 M_2 selections in M_3 generation ranged from 6.3 to 25.4 while that of control grown along with ranged from 8.7 to 20.8 with a mean value of 18.2.Despite a wider variation observed for grain yield in M_2 and M_3 generations the positive shift observed in harvest index is very narrow.

SUMMARY

A variety of pigeonpea with medium maturity was gamma irradiated at 15,20,25,30,35,and 40 kR Populations derived from 20,25,30 and 35 kR treatments were studied in M_2 and M_3 generations and mutations affecting maturity period,plant architecture and grain yield were isolated.Based on chlorophyll mutation frequency and survival 25 kR gamma ray treatment was found most effective in the variety used.Irradiation generated wider variability for several characters affecting grain yield and plant type.One among the early flowering mutants isolated was a month earlier to the mother variety.Few high yielding plants identified in M_2 and M_3 generations had altered plant type and slightly improved harvest index.Very low intergeneration relationship was observed for yield and harvest index.

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TABLE 1. Germination and survival of gamma ray treated seeds of pigeonpea, variety Hy-2

Treatment	Number of seeds sown	Number of seeds germinated	% Germination	Plants survived*	Morphological variants
Control	300	269	89.7	214	-
15 kR	300	226	75.3	157	-
20 kR	900	519	57.7	232	-
25 kR	900	387	43.0	194	-
30 kR	900	225	25.0	118	-
35 kR	900	105	11.7	67	-
40 kR	300	8	2.7	-	-

* Part of M_1 plants were lost due to Fusarium wilt.

TABLE 2. Frequency of chlorophyll deficient mutations induced by gamma ray in pigeonpea, variety Hy-2

Treatment	Number of				Chlorophyll deficient mutants per 100	
	M_1 plants	M_2 seedlings	M_1 plants segregating	M_2 mutant seedlings	M_1 plants	M_2 seedlings
Control	100	3298	0	0	0	0
15 kR	157	3249	5	19	3.18	0.58
20 kR	232	5264	29	57	12.50	1.08
25 kR	194	3873	32	58	16.49	1.50
30 kR	118	2314	18	38	15.25	1.64
35 kR	67	1543	9	18	13.43	1.17

TABLE 3. Gamma ray induced flowering duration variability in M_2 generation

Days to flowering	Number of plants				
	Control	20 kR	25 kR	30 kR	35 kR
70 - 75			1		
76 - 80			5		
81 - 85			0		
86 - 90			2		
91 - 95		10	5	3	11
96 -100	285	106	479	43	189
101 -105	861	842	1073	575	429
106 -110	235	1788	621	591	180
111 -115	70	176	146	125	98
116 -120	126	154	71	26	20
121 -125	20	69	45	23	4
126 -130		13	1	14	2
131 -135		10	4	7	6
136 -140		34	4	7	4
141 -145		9	0	6	2
146 -150			3	3	
151 -155			3		
Total :	1527	3211	2475	1423	945
Range :	97-122	93-144	70-152	94-149	93-144
Mean :	103.8	107.7	104.0	107.2	104.8
S.E. :	0.12	0.11	0.19	0.16	0.21
C.V.(%) :	4.65	5.63	8.97	5.76	6.10

TABLE 4. M_3 Generation yield performance of gamma ray induced early maturing mutants in pigeonpea variety, Hy-2

Pedigree	Days to flower	Number of plants	Mean plant weight(g)	Mean seed weight(g)	100-seed weight(g)	Harvest index
25-161- 9	73	34	104.9	23.1	11.9	22.0
25-163-15	77	31	94.4	22.2	9.8	23.6
25-161- 5	78	42	60.9	13.6	10.7	22.3
25-101- 5	78	18	77.2	18.9	8.0	24.5
25-101- 6	79	38	83.7	19.5	8.0	23.3
25- 62- 5	86	37	152.9	21.5	11.0	14.0
25- 60-15	88	22	125.4	23.6	12.7	18.8
Control	102	150	140.8	26.2	12.1	18.2

TABLE 5. Gamma ray induced variation in single plant yield in M_2 generation in pigeonpea, variety Hy-2

Grain yield(g)	Number of plants				
	Control	20 kR	25 kR	30 kR	35 kR
1- 10	102	991	277	161	113
11- 20	266	1004	531	225	206
21- 30	368	561	493	276	212
31- 40	237	223	403	244	117
41- 50	225	89	247	198	119
51- 60	153	44	145	130	62
61- 70	91	20	82	76	44
71- 80	56	4	48	54	22
81- 90	57	3	22	31	11
91-100	37	0	13	28	9
101-110	13	0	12	7	0
111-120	7	1	9	7	2
121-130			2	7	1
131-140			1	0	2
141-150				0	1
151-160				2	1
161-170				1	
170-180				1	
Total	1612	2940	2285	1448	922
Range	1-120	1-120	1-135	1-173	1-155
Mean	38.45	17.59	30.95	37.12	32.00
S.E.	0.57	0.23	0.42	0.64	0.71
C.V.(%)	60.08	71.97	65.60	65.85	67.74

TABLE 6. Variation for the number of seeds per pod in gamma irradiated M_2 progenies of pigeonpea, variety Hy-2

Number of seeds per ten pods	Number of plants				
	Control	20 kR	25 kR	30 kR	35 kR
15 - 17		46	13	11	6
18 - 20	2	40	12	18	12
21 - 23	15	77	44	35	18
24 - 26	16	131	64	58	31
27 - 29	28	187	110	91	44
30 - 32	58	328	185	133	65
33 - 35	220	564	315	160	93
36 - 38	464	886	683	325	181
39 - 41	486	539	624	383	267
42 - 44	202	148	206	161	158
45 - 47	22	21	40	48	32
48 - 50	14	13	12	13	6
51 - 53		1	3		1
Total	1527	2981	2311	1406	913
Range	18-48	15-51	15-52	15-50	15-51
Mean	37.9	34.7	36.4	37.0	37.7
S.E.	0.11	0.11	0.11	0.10	0.19
C.V.(%)	10.9	16.2	14.7	10.2	15.4

TABLE 7. Gamma ray induced M_2 generation variability for primary branches in pigeompea, variety Hy-2

Number of primary branches	Number of plants				
	Control	20 kR	25 kR	30 kR	35 kR
0	4		5		2
1 - 3	11	28	21	3	4
4 - 6	33	101	93	78	17
7 - 9	107	328	201	121	45
10 - 12	169	393	270	128	107
13 - 15	104	311	219	116	101
16 - 18	22	42	88	80	36
19 - 21		6	21	23	8
22 - 24		1	2	0	
25 - 27				1	
Total :	450	1210	920	550	320
Range :	0-18	1-24	0-22	3-25	0-21
Mean :	10.5	10.5	11.0	11.3	11.9
S.E. :	0.16	0.10	0.13	0.18	0.20
C.V.(%) :	32.3	31.8	36.2	38.0	30.3

TABLE 8. Gamma ray induced M2 generation variability for secondary branches in pigeonpea, variety Hy-2

Number of secondary branches	Number of plants				
	Control	20 kR	25 kR	30 kR	35 kR
0	86	291	196	77	54
1 - 5	159	359	259	121	84
6 - 10	70	250	207	140	84
11 - 15	68	130	133	118	45
16 - 20	42	47	73	42	33
21 - 25	13	18	31	26	11
26 - 30	9	10	16	15	5
31 - 35	3	4	3	6	4
36 - 40		0	1	4	
41 - 45		1	0	1	
46 - 50			1		
Total :	450	1210	920	550	320
Range :	0-35	0-41	0-48	0-41	0-35
Mean :	7.4	5.4	7.4	9.4	9.0
S.E. :	0.29	0.18	0.24	0.34	0.35
C.V.(%) :	84.5	113.8	96.8	84.3	69.9

IMPROVEMENT OF PROTEIN QUALITY IN GRAIN LEGUMES
An overview on mutational improvement of protein quality in pigeon pea

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Abstract

Grain legumes provide an essential part of the diet in developing countries in terms of protein. Besides increasing production also improving the grain quality would be an important objective. The paper discusses the methodology for protein improvement in seeds of pigeon pea.

Introduction

Why Improvement of Grain Legume Protein Quality: Grain legumes are the primary high protein food in several developing countries where diet is largely cereal or tuber based. Cereal protein and legume protein are nutritionally complementary. A protein sufficient diet wherein cereal and legume contribute protein in 70:30 ratio is nutritionally adequate (Hulse, 1975). In Africa and Near East this ratio is 75 cereal to 25 legume, whereas in South East Asia it is 90:10 which is the lowest. Present disparity in the productivity of cereals and legumes and the consequent high cost of legumes in regions of low legume intake deny a nutritionally ideal cereal-legume diet to low-income groups. Under such situations nutritive status of cereal and legume dietary components assumes greater importance.

Considering the legume component, it could be said that many of the grain legumes are inferior per se to major cereals in nutritive value, notwithstanding their high protein and lysine content. Although the seed protein content of legumes is twice or thrice higher than in cereals, the nutritional coefficient of protein content is only marginally better than that of cereals (Meiners and Litzenberger, 1972). Inadequacy of sulphur amino acids, methionine and cystine, in seed protein is the major cause of nutritional inferiority of legumes (Swaminathan, 1967). Tryptophan is also limiting in legumes like pigeon pea. Although improved productivity may lead to increased availability of legume protein in diet, improvement in the level of sulphur amino acids in legume protein is nevertheless important to enhance its nutritive value. Another reason to improve sulphur amino acids in legume protein is the limitation of these amino acids in cereals like sorghum and tubers like cassava, yams and potato, which are the staple food for millions of people in India and Africa.

How to improve protein quality in grain legumes: There could be two possible approaches to improve legume protein quality through increased availability of sulphur amino acids.Firstly, an increase in protein content is likely to increase the amount of these amino acids in seed.This increase,however,may not be nutritionally advantageous because only a positive change in the concentration of sulphur amino acids in protein can improve its biological value.Possibility that an increase in protein content may decrease the concentration of sulphur amino acids in protein also appears real(Adams,1972;Jambunathan and Singh,1980).It is suspected that protein synthesised after a threshold limit is inferior in sulphur amino acids.Second approach is genetic alteration of protein composition through an increase of protein components rich in sulphur amino acids at the expense of those poor in these amino acids.Globulins form the predominant protein fraction (about 85%)of legume seed protein,the rest being albumins.Contradictory results are reported on the level of sulphur amino acids in albumins(Kelly,1972;Bajaj,1972).Hence it is not clear whether quality of legume protein could be improved by shifting the proportion of albumins and globulins.Globulins are composed of two or three major components with different sedimentation coefficient values,namely,2-3S,7-8S and 11-12S. The latter two components are ubiquitous and most abundant. Between these two,the 7-8S component is about one third of 11-12S component(Basha,1974) and has the poorest sulphur amino acid profile(Baily and Boulter,1966;Kelly,1972).Low concentration of these amino acids in proteins does not appear to be due to their limited availability in free amino acid pool during protein biosynthesis(Boulter,1972).Hence mutational alteration of protein biosynthesis at regulatory level may effect the desired shift in the ratio of high sulphur proteins to low sulphur proteins to result in seed proteins with increased sulphur amino acids and nutritive value.

Screening methods to isolate high sulphur amino acid genotypes: Programme to improve sulphur amino acid content in legumes requires chemical methods to screen large populations of breeding material.Success of such a programme therefore largely depends on the availability of a screening method which is rapid, precise to sense smaller differences and less costly.It is also important that the method should employ smaller sample size to permit assessment of variability in early generations.Lack of such methods to estimate methionine and cystine is the major hinderance hampering progress in the improvement of legume seed protein quality.According to the available methods methionine is estimated by ion-exchange chromatography(Moore,1963),gas-liquid chromatography(Finlayson and Mac Kenzie,1976;Mac Kenzie,1977),

thin-layer chromatography(Ferenezi,et al.,1971),microbiological assay(Kelly,et al.,1971) and colorimetric assay(Gehrke and Ussary,1969;Ussary and Gehrke,1970) and cystine by ion-exchange chromatography(Moore,1963),hydrazinolytic method(Goa,1961), kinetic assay(Zahler and Cleland,1968) and colour assay(Felker and Waines,1978).None of these methods are satisfactory to serve a breeding programme.However,some of the methionine assay methods are relatively simpler and merit details.A high positive relationship existing between contents of methionine and cystine in legume protein(Adams,1972;Jambunathan and Singh,1980) is important in this context.A measure of methionine content alone would therefore be sufficient as a preliminary screening.

Important methionine assay methods:

1. Ion-exchange chromatography(Moore,1963) is accurate,reproducible and the standard method,but very slow and costly.
2. Colorimetric assay(Gehrke and Ussary,1969) adapts Mc Carthy and Sullivan colour reaction of autoclaved,hydrolysed,or papain digested samples with sodium nitroprusside.This method is cheaper and be made rapid by automation(Ussary and Gehrke, 1970),but less precise and reproducible.
3. Microbiological assay (Kelly,et al.,1971) is a rapid adaptation of Ford s Streptococcus zymogenes assay for available methionine. This method is fast and cheap,but less precise and reproducible. It also requires the analyst engaged in purely chemical assay to master a different type of procedure.
4. Gas-liquid chromatography (Finlayson and Mac Kenzie,1976) measures methyl thiocyanate after treatment of seed meal with cyanogen bromide.This method is precise,simple and rapid but involves high initial cost and regular handling of toxic chemicals.However,among available methods,this has the potential of better acceptibility.

Total sulphur content is also being used as a screening parameter for total sulphur amino acid content because of a positive correlation existing between them(Porter,et al.,1972;Sandhu,et al.,1974;Evans and Boulter,1974;Blanchar,et al.,1965).However, percentage of protein sulphur in total sulphur is reported to be low in certain legumes(Bhatty,et al.,1977;Jambunathan and Singh, 1980).It is therefore necessary to establish satisfactory relationship between total sulphur and sulphur amino acids in a legume species before choosing total sulphur as a screening parameter.Unlike the methionine assay methods,although tedious, total sulphur estimation is widely used because it is easy to adapt with limited facilities and provides reasonable accuracy and speed.Speed could also be increased by automation(Mottershead, 1971;Basson and Bochmair,1972).

Mutational Improvement of Protein Quality in Pigeonpea

India has the largest share of natural variability available in pigeonpea.Genetic variabilty for sulphur amino acids in this crop is very narrow(IARI,1971) and inadequate for an improvement programme.Artificial mutagenesis is the only alternative to enlarge the variability and improve protein quality in pigeonpea. Total sulphur is a good parameter to monitor the variability in total sulphur amino acids in this crop(Jambunathan and Singh, 1980).Preliminary results obtained on screening mutated populations for total sulphur content is reported here.

Material and Methods

Variety Hy-2 was irradiated at 20,25,30 and 35 kR (Bala Ravi,1981) and M_2 populations were screened for total sulphur content. Pigeonpea in India is consumed as split cotyledons,"dhal",after removing seed coat.Analysis was therefore performed on dhal samples.Total sulphur was estimated according to a modified wet digestion method adapting Blanchar,et al.(1965) and Tabatabai and Bremner(1970):

Soak 2g seed in water for 20 h and then dry at 60°C.Remove seed coat of dried seeds in a decorticator.Mill the dhal in a Cyclotec mill to pass through 0.4mm mesh.Store dhal meal at 30°C to equilibrate moisture level.Weigh 0.2g sample in to a digestion tube containing two glass beads,add 3 ml con.HNO_3(Analar grade) and allow to stand overnight.Heat the tubes at 150°C for 1 h in a Tecator digestion block where 40 samples are simultaneously handled.Allow tubes to cool at room temperature for 15 min and then add 2 ml perchloric acid(70%,Analar grade).Heat the tubes again at 235°C for 1 h 30 min,with an initial slow and gradual rise of temperature.Cool tubes to room temperature,add 1 ml 6N HCl(Analar grade) and heat at 150°C for 20 min.Cool to room temperature and make volume to 75 ml mark with distilled water. Treat an aliquot of 30 ml digest with 0.3g $BaCl_2$ crystals (Analar grade and crystals graded between 25 and 40 mesh) and shake for one minute on a vortex mixer.Measure the developed turbidity at 420 nm using Spectronic-20 spectrophotometer. Adjust zero absorbance using blank digest which did not have sample.Compute total sulphur in sample from a calibrated standard curve using K_2SO_4(Analar grade).It is necessary to run a set of standards along with each batch of samples.

Results and Discussion

Results of total sulphur estimated in part of the M_2 generation material are presented in the Table.Total sulphur expressed as percentage of meal in untreated population ranged from 0.142 to

TABLE: Gamma ray induced variability for total sulphur content in pigeonpea, Variety Hy-2

Total Sulphur (ug sulphur per 200mg meal)	Number of Control/M_2 Plants Screened				
	Control	25 kR	30 kR	35 kR	Pooled
200		1			1
230		3	9		12
260		3	22		25
290	7	200	25	1	46
320	27	36	97	10	143
350	49	59	121	5	185
380	53	72	132	4	208
410	35	101	129	1	231
440	6	38	75	2	115
470		9	15	1	25
500			1		1
Total:	177	342	626	24	992
Range:	285 - 450	195-480	230-500	275-460	195-500
Mean :	366.9	378.2	370.7	355.0	372.9
S.E. :	2.60	2.00	2.02	9.30	1.57
S.D. :	34.28	37.03	50.55	45.66	49.47
C.V.(%):	9.34	9.79	13.60	12.86	13.26

0.225%. In irradiated populations an increased range and coefficient of variation for total sulphur was observed. Mean total sulphur contents of treated and untreated populations, however, did not differ much. Twentysix M_2 plants showed sulphur content above the upper range of control population and one among them had 0.25%, the highest sulphur content. Analysis of protein content in these material is not yet complete. It is not clear at this stage whether the increased sulphur content is the result of increased protein or increased concentration of sulphur amino acids in the protein. About 25% of total sulphur in pigeon pea seed is reported to be from non-protein sources (Jambunathan and Singh, 1980). Therefore it is important to understand the source of increased sulphur in the mutants. These aspects of the study would be reported elsewhere. Results presented indicate that variability for sulphur content could be enlarged through mutagenesis and mutations effecting increased sulphur amino acids could be isolated on screening a larger population.

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VARIETAL IMPROVEMENT IN GROUNDNUT AT BARC

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Abstract

Mutation research on groundnut to improve varietal characters is in progress since 1958 at the Bhabha Atomic Research Centre, Bombay. Till 1978 about seventy mutants were isolated affecting almost all parts of groundnut plant (1, 2). Most of these mutants were induced in the Spanish Improved variety after X- and γ-irradiations. Some were also induced by EMS. These induced mutants formed a 'genetic pool' to develop improved Trombay Groundnut (TG) varieties.

Introduction

TG-varieties Maintain High Yielding Performance:

In the first Research Co-ordination Meeting held at Kuala Lumpur in Malaysia it was reported that the Trombay Groundnut (TG) varieties viz., TG-1, 3, 14, 16, 17 and 19 were high yielding on the demonstration farms (3). However, the large kernel varieties, TG-1, 16 and 19, had a disadvantage of poor kernel development under rainfed cultivation. TG-3, 14 and 17 with medium kernel size were, therefore, preferred for further trials in the 'minikit' experiments (adaptive trials) conducted by State Departments of Agriculture. Yield performances and morphological attributes of TG-3 and 14 being similar, TG-3 and 17 only were tested during 1978-80. Results reported by the State Departments of Agriculture of Andhra Pradesh, Karnataka and Orissa (covering about 30% of groundnut area in India) are summarised in Table I. Comparative yields obtained in different seasons showed that both

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the varieties maintained their high yielding performances in the minikit trials also.

Spreading Type 'Sequential' Flowering Cultures Developed:

Developing a desirable plant structure would contribute to increased productivity. Being geocarpic fruit, pod formation in groundnut requires penetration of gynophores (pegs) into the soil. Therefore, a plant type which allowed maximum number of pegs to enter soil would be highly desirable. In this respect groundnut varieties with spreading/trailing growth habit are well suited for maximum production provided all the nodes on the primary branches produced inflorescences leading to pod production.

Spreading growth habit is a characteristic of Virginia group of groundnut varieties (4). However, the branching and flowering pattern being 'alternate' (normally two vegetative and two reproductive nodes in succession on primary branches) maximum potential for production is not reached in the present cultivars. Efforts to obtain plants with maximum pod setting potential through 'sequential' flowering pattern are in progress in the U.S.A. also (5,6).

TG-17 and 18A were crossed in 1977-78 to a high yielding national variety, M-13, which has spreading habit of growth and 'alternate' flowering pattern. In the segregating populations in F_2 and F_3 generations, plants having spreading habit but 'sequential' flowering pattern were isolated. Genetic segregations on the flowering pattern and comparative pod production potentials in these 'sequential' flowering spreading cultures were studied during 1979 and 1980. Summaries of these observations are given in Tables II and III. Segregation studies on flowering pattern in the cross, TG-18A x M_{13}, showed (Table II) that the 'sequential' flowering pattern was controlled by two recessive duplicate factors. Similar studies in another cross, TG-17 x M-13, are in progress.

Plants having spreading and 'sequential' flowering pattern with varying kernel sizes (0.5 to 1.0 gm per seed) were selected for improved pod setting potential. Their plant progenies bred true for growth habit and flowering pattern in F_4. One of the progenies (Sel.5-2) had uniform pod size and increased pod setting potential. Comparative characteristics of this progeny in F_5 are given in Table III. The selection resembled M-13 in respect of growth habit and leaflet size; while its number of branches and number of leaves were similar to those of TG-3. The number of pods, pegs, nodes producing pods and pegs on the branches at 100 days were maximum in the new plant type indicating high production potential. Selections for different pod sizes and other combination of characters are being studied further.

Spanish Type Cultures with Large Pods:

Increasing pod size without decreasing the number of pods per plant in a cultivar is another way of improving yield. Combining large pod size and early maturity would help in developing an improved variety, and ensure more uniform development of large kernels which would be preferred by export trade.

Large pod character in groundnut is associated with Virginia group (4) of cultivars, which are distinguishable by 'alternate' flowering pattern, profuse branching and seed dormancy. Transferring this large pod character to the Spanish and Valencia group of cultivars having early maturity and generally high shelling out turn, was not successful in the past (7).

In an experiment to induce further mutations in the TG-varieties a new plant type was obtained in TG-18 after 20 kR γ-irradiation. This culture had 'sequential' flowering pattern (8) as in the Spanish Improved (SP) and TG-17, unlike profuse branching and 'alternate' flowering pattern in TG-18 and TG-1 parents. Similar cultures (TG-16 and TG-19A) with large pods and early maturity were also isolated in other

crosses viz., TG-1 x Virescent and TG-17 x TG-1. Comparative studies made in Kharif 1980 are summarised in Tables IV and V. These observations showed that plant height was generally reduced in all the three cultures compared to that of SP. Other morphological characters excepting pod size were similar. Pods and kernels were as large as in TG-1. Unlike in TG-1 the kernels had split seed coat. In respect of shelling out turn and HPS (Hand Picked Selection grade) large kernel recovery, the new cultures resembled TG-18. Since the oil contents were lower compared to that of SP, the kernels of new cultures were better suited for "table purpose" and hence HPS export. TG-16 and TG-19A are included in the national evaluation experiments of the ICAR.

Breeding for Earliness:

Early maturity is helpful especially in developing multiple cropping pattern under irrigated farming systems. For this purpose maturity of less than 100 days is desirable.

Among the present cultivars maturity period varies from 110 to 120 days in 'bunch' types and 125 to 145 days in spreading. Irrigated groundnut cultivated during December-April (Rabi season) is becoming popular in Southern and Central parts of India. Bunch varieties are invariably grown in this season to save on irrigations. The earliest maturing culture in groundnut is Chico (9) which matures in about 90 days. However, due to small kernel size (0.30 gm/seed) it is poor yielding. Recently another culture viz., JL-24 (Phule Pragati) having normal pod size, is reported to be maturing in 90 days at Jalgaon, Maharashtra (10). Two early maturing cultures viz., TGE-1 and TGE-2 were developed by crossing Tall mutant with TG-9 and Gujarat dwarf with TG-3 respectively in 1976. All these four cultures were included along with two popular cultivars, SB XI and TG-3, to study the development of pods and kernels to understand the stage of maturity.

The observations made at 10 days interval from 85 days to 115 days during Kharif 1980 are summarised in Table VI. Moisture losses in the pods of different cultures decreased to about 40% at maturity.

Accumulation of dry matter in the pods during 85-115 days showed a linear increase in the late maturing varieties; while it levelled off after 95 days in the early cultures (Fig. 1). The number of pods in both the groups were similar. These indicated that the dry matter accumulation and maturation were faster in the early cultures. Shelling out turn was generally optimum at 95 days in the three early cultures unlike 105 days in others. Similarly weight of 100 kernels was also optimum at 95 days in the early cultures; while in others it took 115 days to reach the optimum size. Studies on the oil content in kernels also reflected similar relation with maturity. Thus the studies demonstrated that the shelling out turn and 100 kernel weight at a given time would indicate stage of development. Accordingly TGE-1 and TGE-2 are maturing along with Chico in less than 100 days while TG-3 and JL-24 matured at 115 days. Inspite of improvement in seed size compared to Chico, TGE-1 and 2 could not compete in yield (Table VI) with high yielding TG-3. Therefore, further studies to improve seed size in the early cultures are in progress.

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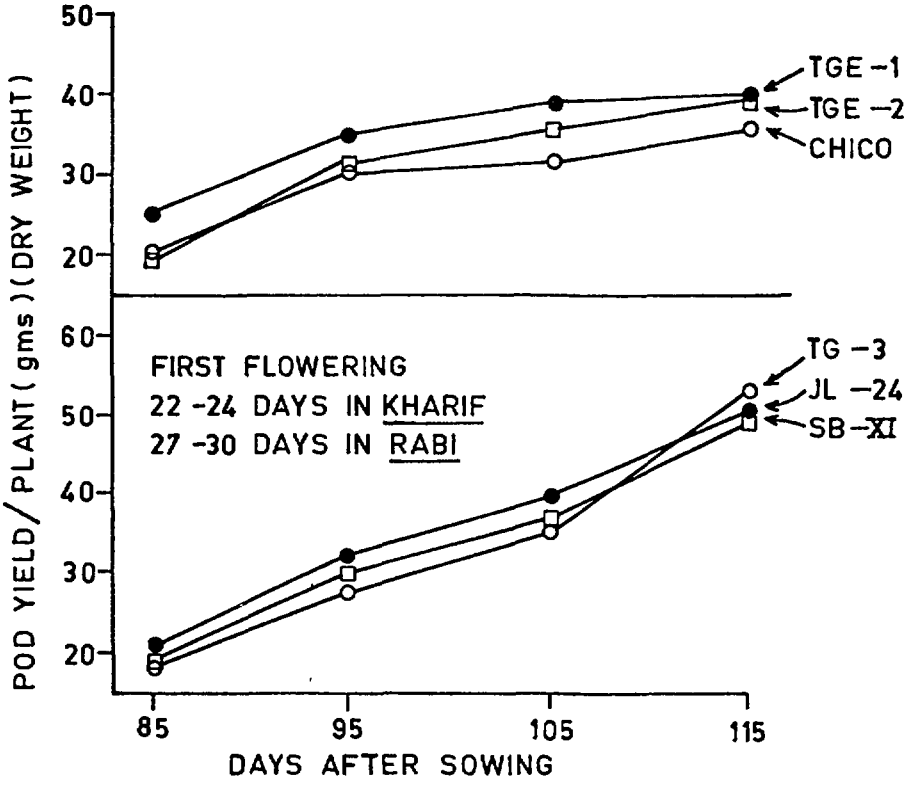

Table I Summary of Minikit Results Obtained During 1978-80

States	Season	Number of District	Number of Trials	Yield in kg/ha TG-3	Check	% difference
Andhra Pradesh	*Rabi 1978-79	5	69	635	694	- 9
	Kharif 1979	9	53	1007	817	+ 23
	Rabi 1979-80	3	42	1835	1678	+ 10
	Kharif 1980	5	17	905	784	+ 15
				TG-17	Check	
	*Rabi 1978-79	5	14	594	654	- 10
	Kharif 1979	6	17	737	633	+ 16
				TG-3	Check	
Karnataka	Rabi 1978-79	14	70	1719	1472	+ 17
	Kharif 1979	5	40	834	540	+ 54
	Rabi 1979-80	5	60	1800	1470	+ 22
	Kharif 1980	7	53	1295	1078	+ 20
				TG-17	Check	
	*Rabi 1978-79	14	58	1592	1672	- 5
	Kharif 1979	3	20	663	492	+ 35
	Rabi 1979-80	5	166	1908	1612	+ 18
	Kharif 1980	2	48	846	631	+ 34
				TG-3	Check	
Orissa	Rabi 1978-79	1	16	1272	919	+ 38
	Kharif	1	20	1714	1065	+ 60

*In Rabi 1978-79 the seed rate in TG-varieties was lower than in locals especially in Andhra Pradesh and Karnataka.

Table II

Segregation for Growth Habit and Flowering Pattern

Cross	F_1	Generation	No. of progenies	Segregation frequency				Phenotypic ratio for AF:SF	X^2	P value
				AF		SF				
				Spr	B	Spr	B			
TG-18A x M-13	M-13 type	F_2 (1979)	2(AF,Spr)	72	9	5	-	15:1	0.0285	85-90
		F_3 (1980)	16 (")	215	-	-	-	-	-	-
			20 (")	315	144	-	-			
			19 (")	162	83	13	6	15:1	0.354	50-75
			17 (")	118	95	36	36	3:1	0.388	50-75
			4 (AF,B)	-	58	-	4			
			5 (B)	-	90	-	-			
			3 (SF,Spr)	-	-	38	10			
			2 (")	-	-	32	-			

AF = Alternate flowering; SF = Sequential flowering; Spr = Spreading; B = Bunch.

Table III

Comparative Characteristics and Pod Setting Potential of Sel-5-2

	Spacing (cm)	Ht. (cm) Stem/primary br.	Branches	Total nodes			Pods		Total pegs
				Leaves	Fruiting	Pegging	D	UND	
TG-3	25	39/43	6+9	224	43	86	54	38	189
	15	34/38	6+4	160	35	-	39	32	102
TG-17	25	31/38	5+1	140	41	62	49	34	194
	15	27/34	4+1	100	35	-	38	25	90
TG-18A	25	32/40	6+4	200	50	103	50	53	232
	15	30/35	4+2	120	40	-	29	38	134
M-13	25	20/46	10+26+12	492	30	93	30	17	185
	15	19/38	7+20+4	300	20	-	13	12	60
Sel-5-2	25	23/64	7+4	248	76	135	115	24	303
	15	19/50	5+4	200	50	-	50	18	170

D - Developed UND - Underdeveloped

Table IV

Comparative Characteristics of Cultures Having 'Sequential' Flowering Pattern and Large Pods

Characters	Parents			Improved cultures		
	SP	TG-1	TG-18	TG-16	TG-18A	TG-19A
Plant height (cm)	85	71	73	70	70	75
Branch number (Primary + Secondary)	6+7	11+23	8+17	6+6	5+4	5+4
Foliage colour	Green	Dark Green	Green	Green	Green	Dark green
Leaflet (cm)						
Length	7.5	6.2	6.2	6.8	6.2	7.4
Breadth	3.5	3.3	3.0	3.4	3.0	3.4
Shape	Oblong	Elliptic	Elliptic	Oblong	Elliptic	Oblong
Flowering pattern	S	A	A	S	S	S
Pod number (1+2 kernels)	6+40	6+30	8+36	8+32	5+32	6+30
100 kernel wt. (gm)	50	85	110	85	105	95
Dormancy (days)	Nil	60	25	15	15	25
Seed Coat	Normal	Normal	Split	Split	Split	Split

Alternate = A; Sequential = S.

Table V

Economic Attributes

Variety	Yield (gm/plant)	Shelling %	Days to maturity	Oil %	% HPS kernels with 100 seed wt. (gm)		
					>90	(90-70)	UD
SP	36	75	115	49.5	-	-	-
TG-1	36	70	135	47.4	26	38	28
TG-18	40	75	125	46.0	56	25	19
TG-16	48	74	125	47.7	46	30	24
TG-18A	42	76	120	45.9	55	28	17
TG-19A	46	72	125	47.0	55	29	16

UD - Underdeveloped

Table VI

Earliness Influencing Kernel Development and Maturity in Groundnut (Kharif 1980)

Cultures	Days to harvest	Wt. of pods /plant (gm)		% Moisture loss	% Shelling	100 kernel wt.	% oil content
		Wet	Dry				
SB XI	85	44	18	59	68	24	46.2
	95	56	30	46	72	32	48.4
	105	60	35	42	75	37	50.3
	115	71	45	37	75	38	50.5
TG-3	85	56	16	72	64	24	45.9
	95	60	25	68	69	35	47.4
	105	75	36	52	72	42	50.8
	115	83	53	36	73	48	51.0
JL-24	85	66	21	68	65	24	44.4
	95	70	34	51	70	38	47.0
	105	75	37	48	72	45	48.6
	115	84	51	39	73	50	50.0
Chico	85	46	20	57	72	28	47.0
	95	56	30	46	76	30	49.6
	105	58	33	43	77	29	50.0
	115	57	36	37	77	30	50.3
TGE-1	85	55	19	65	71	28	48.0
	95	59	32	46	74	32	49.5
	105	60	36	40	77	35	51.5
	115	63	40	37	77	36	51.7
TGE-2	85	60	25	58	72	35	48.0
	95	63	35	44	75	38	49.5
	105	64	38	41	77	40	50.0
	115	62	40	35	76	39	51.3

INDUCED MUTATIONS IN GRAIN LEGUMES

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Abstract

Mutation induction experiments on various grain legume species are carried out at IARI since a number of years, all aiming at improving the germ plasm available for the development of better varieties. The paper describes the promising results in chickpea, lentil, pea and cowpea.

Introduction

The present report contains the results of various yield trials that were conducted with induced mutations of chickpea, lentil, pea and cowpea during the period after the first FAO/IAEA Research Co-ordination Meeting on the use of induced mutations for improvement of grain legumes held at Kuala Lumpur in 1979.

Chickpea: The mutations selected for changed plant type in chickpea were tested in the M_3 generation, in which most of the mutant strains outyielded the respective controls by a considerable margin. The mutants produced a higher number of pods and grains, and have higher protein content, as can be seen from Table 1.

Table 1. Performance of the plant type mutants

Average of	Biological yield per plant, g	Harvest index, %	Pods per plants	Seeds per pod	Seeds per plant	100-grain wt., g	Grain yield per plant, g	Protein content %
Controls (4)	58.5	46.7	160.0	1.22	194.3	12.7	27.3	23.9
Mutants (13)	91.1	45.0	231.3	1.39	306.9	12.8	40.8	25.5

Some of the most promising mutants of chickpea were evaluated in the multilocation trials all over the country during the cropping season of 1979-80. All these mutants were compared with the best check varieties of

Table 2. Performance of gram mutant strains in the All-India Coordinated Gram Initial Evaluation Trial, 1979-80 (zonal mean, yield in q/ha)

Variety	North Plains West	North Plains East	Central	Peninsular
Best check	21.21	19.96	17.12	11.22
BG-401	22.56	-	-	13.12
BG-402	22.53	21.75	-	-
BG-403	-	23.93	19.13	-
BG-404	-	21.43	20.39	-
BG-405	23.16	-	-	14.13
BG-406	-	-	19.21	12.34

the respective zones. Six of these mutants gave better yields than the standard checks. The results of these trials are presented in Table 2.

Lentil. Mutation experiments were carried out in lentil varieties belonging to the two distinct taxonomic groups, microsperma and macrosperma. It was found that the genotypes in the macrosperma group were more responsive to mutagenic treatment and gave a higher frequency of mutated progenies in M_2 (Table 3).

A green seeded mutant was isolated, which could become a nobility for the lentil growing regions in the Indian subcontinent, as most of the varieties cultivated in this part of the world have dark, mottled testa. This mutant has been screened for one year in single-row trials and has been found promising in grain yield also. The mutant is being tested at present in replicated trials.

Table 3. Frequency of chlorophyll mutations in lentil varieties belonging to the *microsperma* and *macrosperma* groups (% M_2 progenies mutated)

Treatment	Microsperma				Macrosperma	
	Pusa-1	L-235	L-258	L-259	L-1491	L-1492
Gamma-rays:						
6 kr	7.6	5.8	5.9	4.5	14.6	9.7
10 kr	13.2	11.1	12.9	9.5	21.3	16.3
Average	10.9	8.2	9.2	7.0	18.2	13.1
NMU:						
0.005%	15.2	11.3	13.2	8.9	20.5	24.2
0.01%	22.3	16.7	19.7	15.1	36.8	39.4
Average	18.6	14.1	16.3	12.0	28.6	31.8

Pea. A mutant variety of grain pea (L 116) was developed after seed treatment with ethylene imine. After 3 years Institute Trials, the variety was handed over to the All-India Pulse Coordinated Project. On the basis of coordinated trials in different parts of India, it was recommended for release, and finally released and notified by the Government of India in March, 1979. The variety was released under the popular name *Hans*. The overall performance of this variety in different trials is shown in Table 4.

Table 4. Performance of pea mutant variety L 116 (Hans) in different trials

Trial	Average yield over many years (g/ha)		Superiority over T.163 (%)
	T.163	L 116(Hans)	
Institute Trials	5.4	6.6	+22.4
Late sown trials	2.4	5.1	+112.5
Coordinated Trials	14.8	12.7	-13.3
Agronomy Trials	18.1	19.7	+8.5
Minikit Trials	9.5	9.9	+3.6

In another mutation experiment, an attempt was made to study the induced changes in protein content and quality of pea seed. A large number of mutants with increased and reduced protein content were isolated on the basis of repeated testing over 3 years. Correlation between protein content and a few economically important parameters were worked out (Table 5).

Table 5. Correlation coefficients in the induced mutations of pea

Character	Methionine	Grain wt.	Yield per plant
Protein	-0.6477**	-0.0928	-0.4547**
Methionine		0.0245	0.2737*
Grain weight			0.0350

Methionine content was also included in this comparison. As can be seen from Table 5, the correlation coefficient between protein and methionine content was negative and statistically significant (-0.6477). The protein content was also negatively correlated with grain yield per plant (-0.4547). On the other hand, methionine content showed a mild and statistically significant positive correlation with plant productivity (0.2737). Therefore, the general pattern of yield and protein as well as protein and methionine content, as reported in other crops, was repeated in this case also.

Table 6. Average chemical (amino acid) score of induced high protein mutants in pea based on methionine, cystine and tryptophan

Variety and mutants (averaged)	Protein, %	Chemical score (% of egg protein		
		methionine	cystine	tryptophan
Hen's egg	12.38	100.00	100.00	100.00
P 77 (control)	24.68	49.70	53.78	66.66
P 77 (mutants)	30.85	50.80	57.73	58.03
P 78 (control)	24.08	40.51	59.22	66.22
P 78 (mutants)	29.65	47.82	69.57	63.50
P 91 (control)	23.19	45.57	68.99	61.20
P 91 (mutants)	31.15	48.97	69.58	56.19
P 119 (control)	28.76	40.51	62.70	67.39
P 119 (mutants)	32.53	47.51	64.90	57.10
P 192 (control)	22.51	46.84	91.60	62.88
P 192 (mutants)	27.42	49.57	78.41	64.13

Nevertheless, it was possible to identify mutants having a combination of good yield (sometimes better than the control) and high protein content. Similarly, in spite of negative associations, it is possible to get mutants having a combination of high protein--high methionine levels. This can be seen from the averaged chemical (amino acid) score of the high protein mutants in pea calculated on the basis of methionine, cystine and tryptophan--the amino acids that are particularly important in grain legumes because of a relatively lower level than in cereals (Table 6). There were several instances in which the methionine content also improved along with increase in the protein levels. Therefore, it can be concluded that the negative correlations between various characters can be modified if a large number of mutational variability is generated. The same trend can also be demonstrated for the yield--protein relationship.

Cowpea. Following chemical treatment of an old white-seeded cowpea variety (Pusa Phalguni), a large number of different chlorophyl and morphological-physiological mutations were isolated. The chemicals used for this study were ethylmethane sulphonate and nitrosomethyl urea. A few cases of mutations with unstable behaviour were also recorded.

Table 7. Performance of cowpea mutant varieties in the Coordinated Trials (All-India average)

Variety	Yield, q/ha		
	1977	1978	1979
V 16	9.74	11.93	20.25
V 37	8.90	10.96	19.67
V 38	8.33	9.70	14.47
C-152 (Control)	6.76	9.48	14.60

The genetic instability of these induced variants was confirmed through four generations, in which the whole material was screened on single plant basis.

Ultimately, a very small number (1-1.5) of plants showed varying degree of stability. Such stable variants were assessed for their yield potential. In this process it was found that a large number of these selections were also not constant. However, some constant lines could be isolated, and these were tested for 3 years in Institute Trials. After the initial screening, three most promising varieties (V 16, V 37 and V 38) were entered in the All-India Coordinated Varietal Trials, where they were tested for another 3 years (Table 7).

On the basis of the results of these trials, all the three varieties were recommended by the All-India Coordinated Project for release in April, 1980. These varieties are being tested at present on the farmer's field, and on the basis of results already received it can be expected that these varieties will be ultimately released for cultivation by the Govt. of India.

INDUCED MUTATIONS FOR SOYBEAN IMPROVEMENT*

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Abstract

Seeds of soybean variety Orba were treated with various doses of fast neutrons, gamma rays, EMS (Ethyl methanesulphonate) and NaN_3 (Sodium azide) with the aim to isolate mutants, having early maturing time, adaptation to high altitude cultivation, short stature, lodging resistance and improved yield.

Seventeen lodging resistant mutant lines (confirmed in M_3) were tested in the M_4 and M_5 generations. Short stature and early mutants were significantly shorter and earlier than the original variety.

Some mutant lines have bigger seeds and at least the same yield as the original variety Orba.

Introduction

Food legumes supply a major part of the vegetable protein and oil for the human diet in many countries of the Asia and Far Eastern region.

Soybean (Glycine max (L.) Merr.) is the most important among legume crops in Indonesia. The consumption of soybean in Indonesia is increasing, but the yields are low (the acreage is about 703,878 hectares with the annual production of 517,199 tons and the average yield of about 0.73 t/ha), therefore a large amount of soybean is imported and in 1979 the import amounted to 22 million U.S. dollars, (1, 2).

SOMAATMADJA (3) reported that in Indonesia 95% of soybean was cultivated in the lowland around an altitude of 100 m - 250 m. Growing soybean in the highland is hampered by the lack of adapted varieties.

The development of short-stature, lodging-resistant, early maturing varieties is a common breeding objective and to generate such variability in crop plants by mutation induction, in general, is not difficult (for reviews see 4, 5, 6 and 7). Therefore, the objective of this research is to find such mutants having improved yield capacity under highland conditions.

Materials and methods

Seeds of the soybean variety ORBA were used in this programme. Dry seeds were treated with fast neutrons, gamma rays, EMS (Ethyl methanesulphonate) and NaN_3 (Sodium azide). Fast neutron irradiation was carried out by courtesy of IAEA in the SNIF (Standard Neutron Irradiation Facility) of the ASTRA swimming pool reactor, Seibersdorf, Austria. Gamma irradiation was carried out using a ^{60}Co source at the Pasar Jum'at Atomic Energy Research Centre, Jakarta, Indonesia. Doses and the number of treated seeds used were as follows:

* This work was financed partially by the IAEA Research Contract No. 2231/RB.

Mutagen/Dose		Number of seeds
Fast neutrons	: Control	2,000
	1500 rad	2,000
	2200 rad	2,000
Gamma rays	: Control	10,000
	20 krad	10,000
	25 krad	10,000
EMS *	: Control	2,000
	0,125 %	4,000
	0,25 %	4,000
	0,40 %	4,000
NaN_3**	: Control	2,000
	1×10^{-3} M.	4,000
	3×10^{-3} M.	4,000

* 4 hrs presoaking, 12 hrs treatment, 2 hrs post-washing.
** 2.5 hrs presoaking, 2.5 hrs treatment, 2 hrs. post-washing.

The M_1, M_2 and M_3 generations were grown at the Horticulture Experimental Station in Lembang at an altitude of 1200 m above sea level. For the M_2 generation, one pod from the main stem of good looking (healthy) M_1 plants was randomly harvested. For each fast neutrons and gamma rays dose, 5,000 M_2 plant were grown. From the EMS and NaN_3 treatments, the number of M_2 plant were approximately 3,000 plants/treatment. Visual selection of M_2 plants (including controls) were carried out, followed by single plant progenies in M_3. Selected homogeneous M_3 lines were further tested to evaluate their yield potential in M_4 and M_5 generation in replicated randomized trials (plot size 4 x 2.8 m) under highland conditions at Lembang.

Results and discussions

In the M_2 generation, approximately 35,000 plants were grown as bulk population. Untreated plants were included as check. Observations were focussed on the selection of potentially useful mutants with short stature, lodging-resistance, earliness and improved yield characters. 250 plants with different characters and phenotypically different from the control plants were isolated and raised in the M_3 generation as single plant progeny. Control plants were inserted as every tenth row. Among the progenies, 17 mutant lines looked promising. These were then tested in M_4 and M_5 generations to evaluate their yield potential, maturity and other characteristics in preliminary yield trials (Table 1).

Short stature mutants, 162-NF.22, 181-NF.22, 166-NF.15, 62-GM.20, 227-E.400, 104-E.250, 35-E.125 and 105-SA.10 were significantly shorter than the parent cultivar. Mutants Nos. 162-NF.22 and 62-GM.20 had narrow and thicker leaves. Mutants Nos. 35-E.125, 104-E.250 and 105-SA.10 had broader leaves and out of these, Nos. 104-E.250 and 105-SA.10 had good grain yields. Some of the early mutants also maintained the yield capacity of Orba.

Three mutant lines, Nos. 162-NF.22, 227-E.400 and 104-E.250 have considerably bigger seeds. Bigger seed size was also obtained by PATIL (8) in his mutation breeding experiments with groundnut.

All mutants described were resistant to lodging and looked stable in two generations under highland condition. Adaptability tests of these mutants will be conducted in M_6 generation in different locations.

Table 1. Plant growth and yield potential of variety "Orba" and its 17 mutant lines grown under highland condition

Mutants	Plant height (cm)		Maturity (days)		Yield/plant (g)		Seed weight (g/100)	
	M-4	M-5	M-4	M-5	M-4	M-5	M-4	M-5
Control	67.61	68.80	118	118	26.83	28.14	11.42	10.03
162-NF.22[a]	34.00**	44.63**	98**	98**	23.17*	27.96	13.72**	14.52**
24-NF.22	65.45	67.37	118	118	28.83	27.36	11.48	11.60*
181-NF.22	59.55**	62.80	118	118	26.66	30.76	11.30	10.60
182-NF.22	70.60	73.97	118	118	27.00	27.52	12.08	11.70*
8-NF.15	63.07	65.77	118	118	31.33**	29.85	12.11	12.07**
166-NF.15[c]	61.50*	55.20**	120	120	18.11**	16.11**	10.05	11.40
206-GM.25	71.90	70.30	118	118	30.63**	25.64	12.13	10.92
203-GM.25	66.20	67.07	118	118	32.58**	27.52	11.57	10.83
202-GM.25	68.44	66.97	118	118	32.08**	28.99	11.43	10.27
62-GM.20[a]	39.89**	48.90**	104**	104**	18.17**	20.37**	10.22	10.43
227-E.400	50.45**	48.70**	114*	114*	22.50**	22.17**	12.87*	13.60**
225-E.400	81.23**	80.70**	133**	133**	36.50**	32.61**	11.29	10.17
104-E.250[b]	44.41**	44.73**	108**	108**	29.66*	29.59	19.95**	20.30**
189-E.250	67.45	68.03	118	118	32.50**	29.25	11.97	10.47
35-E.125[b]	36.98**	41.30**	121	121	18.50**	20.77**	10.11	11.90*
116-SA.10	66.70	65.93	118	118	30.75**	27.64	11.42	11.27
105-SA.10[b]	45.25**	46.83**	123**	123**	27.36	31.99**	11.73	10.77

*, ** significantly different from control.

a: mutants with narrow and thicker leaves
b: mutants with broad leaves
c: mutant with more hairy stem and leaves and white flowers

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SOYBEAN PRODUCTION IMPROVEMENT THROUGH INDUCED MUTATIONS

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Abstract

Fast neutrons, EMS and sodium azide are being used in an attempt to induce useful mutations in soybea. Short stature and early growing mutants were selected. A study is also undertaken to select for better symbiotic nitrogen fixation using ^{15}N.

Introduction

The experiment aims at induced mutations to improve the yielding ability of the soybean variety "Orba". Increased variability for components of yield in soybeans by seed treatment with mutagenic agents has been obtained by Conger et al. (1).

The effectivity of treatments used in the experiments was assessed in M_2 field grown populations by recording the number of chlorophyll mutants. The results of this assessement are presented in Table 1. Within the range of doses applied, the frequency of chlorophyll mutants in EMS treatment increased with the increasing concentration. Treatment with sodium azide (NAN_3) did not produce any chlorophyll mutants, but a high frequency was observed in fast neutron treatment (N_f).

A random sampling of 300 control plants was made to establish the frequency distribution for number of pods per plant (Table 2) and seed weight per plant (Table 3) in the control population. This information was used to decide whether to maintain or discard a particular plant selected visually from the M_2 population. With this approach, from a number of visually selected M_2 individuals we only maintain those with either a number of pods/plant greater than 105 or a seed weight per plant of more than 20.0 g or both. Table 4 and Table 5 show the results of this selection. The progeny testing of the selected individuals is being started in the M_3 generation (M_2 progenies) and will be continued through successive generations to attain a fairly high degree of homozygosity before testing on a large scale.

Some attention was also given to the selection for short stature (dwarf and semi-dwarf) and early flowering. The results are presented in Tables 6 and 7. The frequency of early flowering was relatively higher than that of short stature.

It is too early for drawing conclusions with respect to the relative effectivity of treatments. It is interesting to note, however, that a number of plants has been selected for further evaluation from sodium azide treatment although we were not successful to detect any chlorophyll deficiency mutant.

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There is great interest in the possibility of improving symbiotic nitrogen fixation in legumes. The method used by Fried and Broeshart (2) was adopted in an experiment attempting to evaluate the symbiotic N fixed by a number of mutant lines obtained from gamma radiation treatment of 40 Krad. N-15 labelled urea fertilizer of 1% and 4% atom excess was used. The result is presented in Table 8. The highest mg N fixed per plant was obtained in line No. 40-G/63 and line No. 40-G/65, the lowest was observed in "Evans". However, when expressed in percentage of N in plant derived from fixation, the line No. 40-G/57 showed the highest value up to 80.5% which indicates a great contribution of symbiotic nitrogen fixation in this particular line. The N-15 technique obviously offers a useful application in the evaluation of symbiotic nitrogen fixation since it could provide information at a considerable accuracy on the integral amount of N fixed during a certain growing period of interest. The technique is also applicable to both field and greenhouse evaluation.

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TABLE 1. FREQUENCY OF CHLOROPHYLL MUTANTS OBSERVED IN M-2 POPULATION

No.	TREATMENT	No. OF M-2 SEEDS PLANTED	No. OF SURVIVALS	No. OF CHLOROPHYLL MUTANTS	FREQUENCY (%)*
1.	EMS (0.75 %)	2280	1682	3	0.18
2.	EMS (1.00 %)	2385	724	12	1.66
3.	EMS (1.50 %)	2250	1385	27	1.95
4.	NaN_3 (1×10^{-3} M)	1740	1098	-	0.00
5.	NaN_3 (2×10^{-3} M)	1780	606	-	0.00
6.	NaN_3 (3×10^{-3} M)	465	396	-	0.00
7.	N_F (1000 RAD)	375	145	4	2.76
8.	N_F (2000 RAD)	45	15	-	0.00
9.	CONTROL	2295	1236	-	0.00

*) CALCULATED BASED ON THE NUMBER OF M-2 SURVIVALS.

TABLE 2. DISTRIBUTION OF THE NUMBER OF PODS PER PLANT IN THE CONTROL POPULATION

CLASS	NO. OF PODS PER PLANT	DISTRIBUTION (%)
1	15	1.6
2.	16 - 30	25.3
3.	31 - 45	33.7
4.	46 - 60	20.5
5.	61 - 75	9.3
6.	76 - 90	6.0
7.	91 -105	3.6

TABLE 3. DISTRIBUTION OF DRY GRAIN YIELD (GRAM/PLANT) OF THE CONTROL POPULATION

CLASS	GRAIN YIELD PER PLANT (IN GRAM)	DISTRIBUTION (%)
1.	≤ 4.00	19.6
2.	4.01 - 8.00	48.8
3.	8.01 - 12.00	20.4
4.	12.01 - 16.00	9.2
5.	16.01 - 20.00	2.0

TABLE 4. M-2 SELECTION FOR HIGHER NUMBER OF PODS PER PLANT

No.	TREATMENT	No. OF SELECTED PLANTS*	FREQUENCY (%)
1.	EMS (0.75 %)	10	0.59
2.	EMS (1.00 %)	8	1.10
3.	EMS (1.50 %)	11	0.79
4.	NaN_3 (1×10^{-3} M)	9	0.82
5.	NaN_3 (2×10^{-3} M)	2	0.33
6.	NaN_3 (3×10^{-3} M)	1	0.25
7.	N_F (1000 RAD)	2	1.38
8.	N_F (2000 RAD)	2	13.33

*) ONLY PLANTS WITH NUMBER OF FILLED PODS MORE THAN 105 WERE SELECTED

TABLE 5, M-2 SELECTION FOR HIGHER GRAIN YIELD PER PLANT

NO.	TREATMENT	No. OF SELECTED* PLANTS	FREQUENCY (%)
!.	EMS (0.75 %)	3	0.18
2.	EMS (1.00 %)	5	0.69
3.	EMS (1.50 %)	3	0.22
4.	NaN_3 (1×10^{-3} M)	9	0.82
5.	NaN_3 (2×10^{-3} M)	1	0.17
6.	NaN_3 (3×10^{-3} M)	1	0.25
7.	N_F (1000 RAD)	1	1.38
8.	N_F (2000 RAD)	0	0.00

*) ONLY PLANTS WITH GRAIN YIELD PER PLANT MORE THAN 20 GRAM WERE SELECTED.

TABLE 6. M-2 SELECTION FOR SHORT STATURE (DWARF AND SEMI-DWARF)

No.	TREATMENT	No. OF SELECTED PLANTS	FREQUENCY (%) *
1.	EMS (0,75 %)	4	0,24
2.	EMS (1,00 %)	2	0,28
3.	EMS (1,50 %)	1	0,07
4.	NaN_3 (1×10^{-3} M)	1	0,09
5.	NaN_3 (2×10^{-3} M)	2	0,33
6.	NaN_3 (3×10^{-3} M)	-	0,00
7.	N_F (1000 RAD)	-	0,00
8.	N_F (2000 RAD)	-	0,00

*) CALCULATED BASED ON M-2 SURVIVALS

TABLE 7. M-2 SELECTION FOR EARLY FLOWERING

No.	TREATMENT	: No. OF SELECTED PLANTS	FREQUENCY (%)*
1.	EMS (0,75 %)	10	0,59
2.	EMS (1,00 %)	20	2,76
3.	EMS (1,50 %)	5	0,36
4.	NaN_3 (1×10^{-3} M)	2	0,33
5.	NaN_3 (2×10^{-3} M)	2	0,25
6.	NaN_3 (3×10^{-3} M)	1	0,25
7.	N_F (1000 RAD)	-	0,00
8.	N_F (2000 RAD)	-	0,00

*) CALCULATED BASED ON THE NUMBER OF M-2 SURVIVALS

TABLE 8. SYMBIOTIC N-FIXATION OF SOME SOYBEAN MUTANT LINES AS DETERMINED BY THE USE OF N-15

No.	VARIETY/LINE	MG N FIXED PER PLANT	MG TOTAL N PER PLANT*	PERCENTAGE OF N IN PLANT DERIVED FROM FIXATION*
1.	NON-NOD LINE	-	23.27	0.0
2.	EVANS	33.10	62.58	52.9
3.	40-G/63	54.92	91.32	60.1
4.	40-G/65	53.54	87.79	61.0
5.	40-G/9	40.08	63.44	63.2
6.	40-G/59	38.62	56.98	67.8
7.	40-G/57	45.46	56.46	80.5
8.	ORBA	48.81	68.18	71.6

*) THE ROOTS WERE NOT INCLUDED IN THE DETERMINATION

INDUCED MUTATIONS IN PEANUTS *(A. hypogaea)* Breeding objectives, genetic studies and mutagen treatment methods

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Abstract

The peanut is a major oil crop and an important protein source in food or feed (meal) in southeast Asia. It was one of the first crops subjected to mutagenic treatments, with considerable breeding gains and genetic developments. The breeding objectives for peanuts are listed. It is suggested that in view of the very few sources of resistance to major diseases in the cultivated germ plasm collections and in related species, mutation breeding could make significant contributions once proper mass screening approaches are available.

It was shown in a study with ^{14}C-labeled EMS (in carbon 2) that very little of the mutagen reaches the plumule in the treated seeds, about 2% of the total amount in the seed. The mutagen enters by diffusion and efforts to increase its rate should be adopted.

Introduction

The peanut (Arachis hypogaea L.) is a major crop in Asia, with over half of the world production; mostly in S.E. Asia, the main producers being Burma, China, India and Indonesia [8]. Usually, it is considered an oil crop but it should also be regarded as a food (or feed) crop, being a rich source of proteins. The cultivated peanut has 2n=40 chromosomes, but nearly all of the wild species (22 or 40 or more, depending on authors) have 2n=20 chromosomes. There are two subspecies and four botanical (and agronomical) major types in peanuts and very many cultivars and land varieties. At present there are about 11,000 germ plasm collections [22] of peanuts, with ICRISAT and the USDA being the major custodians.

Induced mutations and breeding

The peanut was one of the first crops to be subjected to mutagenic treatments. Gregory and associates in North Carolina [7, 9, 11] directed their research at a better understanding of the mutation process, genetics and breeding of induced mutations and their utilization. The program in Argentina (Pietrarelli, personal communication 1980, 13, 27] was initiated in 1963 and yielded new commercial varieties in Argentina (Colorado Irradiado INTA) and in New Mexico (N. Mex. Valencia C). The research in India led to the development through mutation breeding (directly or by crossing) of promising new lines and cultivars as reported to this group by Patil in 1979 [25], as well as to basic findings [19, 23, 24, 26].

In Israel, chemical mutagens were used to a large extent, as well as gamma-rays. The studies were aimed at genetic and breeding goals, specifically at the induction and the screening methodology of cytoplasmic mutations [1, 2, 4, 16], treatment methods, mutagenic efficiency and effectiveness, differences in varietal sensitivity, etc. [1, 3, 6, 17]. Several promising lines with thinner shells and smaller plants were obtained by various treatments of Virginia type cultivars, but were not released because of earlier, high yielding material developed through classical breeding. Cytoplasmic mutations were induced by both gamma rays and EMS as described by Ashri and Levy [2, 4] and summarized in Table 1. It should also be noted that the trisomic [5] which was induced by acriflavine (and is similar to that induced in Trombay,[19]) is now utilized in cytogenetic studies in several centers (viz. ICRISAT; Raleigh, N.C.; Tifton, Ga; Stephenville, TX).

Norden [22] estimates that it takes 10-20 years to produce a new peanut variety. Therefore, determining the objectives is very important. The objectives of peanut breeding [see also 9, 11, 20, 21, 22] are:

- Larger economic yields
- Yield stability
- More uniform pod maturation
- Seed dormancy in Spanish and Valencia types
- Shorter (or longer) growing season
- Traits leading to greater adaptation to mechanization and processing
- Improved pod characteristics (e.g. size, shelling percentage, attractiveness)
- Improved flavor and texture
- Higher oil and protein content
- Oil composition
- Increased efficiency of N-fixation
- Higher photosynthetic capacity
- Better harvest index
- Tolerance to environmental stresses
- Resistance to diseases (see below)
- Resistance to A. flavus
- Resistance to insects.

Hammons noted [12] that "cultivated peanuts are devoid of qualitative genetic resistance to most of the diseases and pests" described for the USA. Moss [18] concluded that only resistance found in species belonging to the section Arachis can be transferred now. The paucity of sources of resistance in the cultivated species is demonstrated in the following [after Norden, 22] :

Disease	Resistant lines, No.	Remarks
Cercospora leafspot	1	Cercospora arachidicola and Cercosporidium personatum
Puccinia arachidis	3	
Sclerotium rolfsii	Some	Known also as S. sclerotiorum
Diplodia gossypina	Some	-
Cylindrocladium crotulariae	1(2)	-
Aspergillus flavus	2	Not consistent between years, in these lines the seeds are more resistant to colonization, but their pods are more susceptible.
Rosette virus	1	-

In view of the need demonstrated above, given efficient screening methods for resistance, mutation breeding could make a very significant contribution to peanut breeding by inducing and incorporating resistance in commercial varieties. However, one should consider why genes for resistance do not exist in the natural germ plasm: does it imply that the probability of success is very low ?

Uptake of ^{14}C-labeled EMS by peanut seeds

There have been very few studies on the amounts and kinetics of chemical mutagens' uptake and the mutagens localization in the seeds following various soaking treatments [14, 15, 17, 28, 29]. In an effort to ascertain the mode of uptake by the seeds and the amount of the mutagen reaching the plumule, a series of tests was conducted with seeds of two peanut cultivars and two sesame cultivars, using ^{14}C-labeled EMS in carbon atom 2 and with $^{3}H_2O$ [Brandstein, 6].

The amounts of EMS and $^{3}H_2O$ absorbed in 2-12 hr soaking are shown in Figure 1. A comparison of the EMS and $^{3}H_2O$ uptake (Fig. 1) shows that less

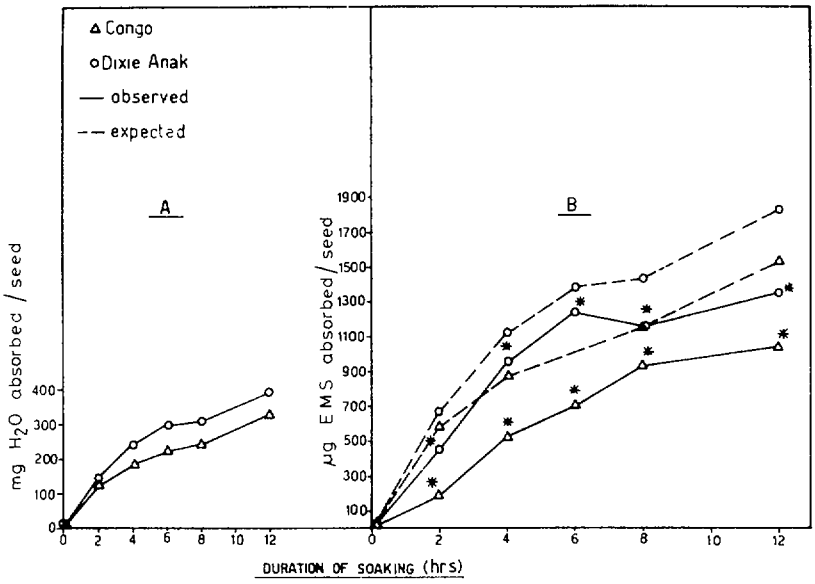

Fig. 1. A comparison of the uptake of tritiated water (A) and EMS (B) in seeds of two peanut cultivars, in relation to the expected uptake (see B). (* indicates a significant difference at the 5% level, between cultivars); from Brandstien, 1980.

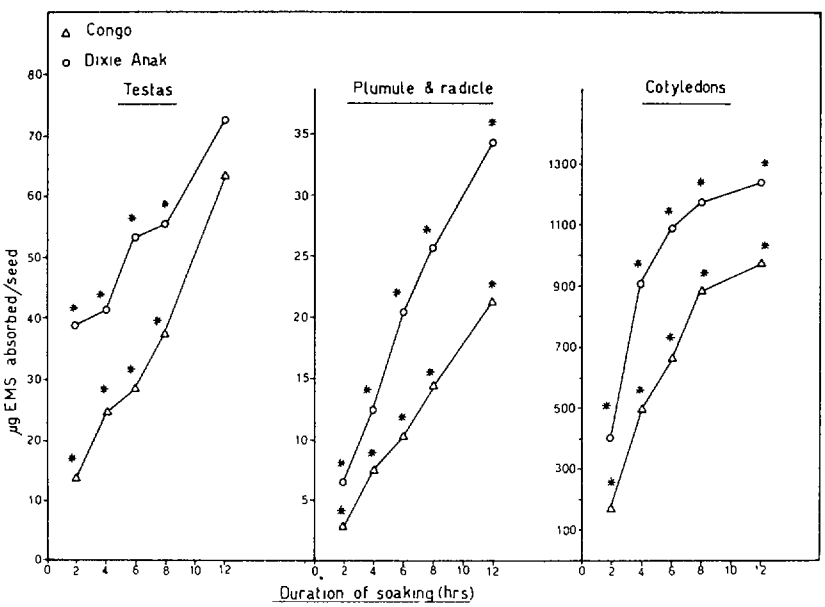

Fig. 2. The effect of the duration of soaking in EMS on the amounts (µg) of the ^{14}C label in different parts of the seeds (* indicates a significant difference at the 0.05 level between cultivars); from Brandstien, 1980.

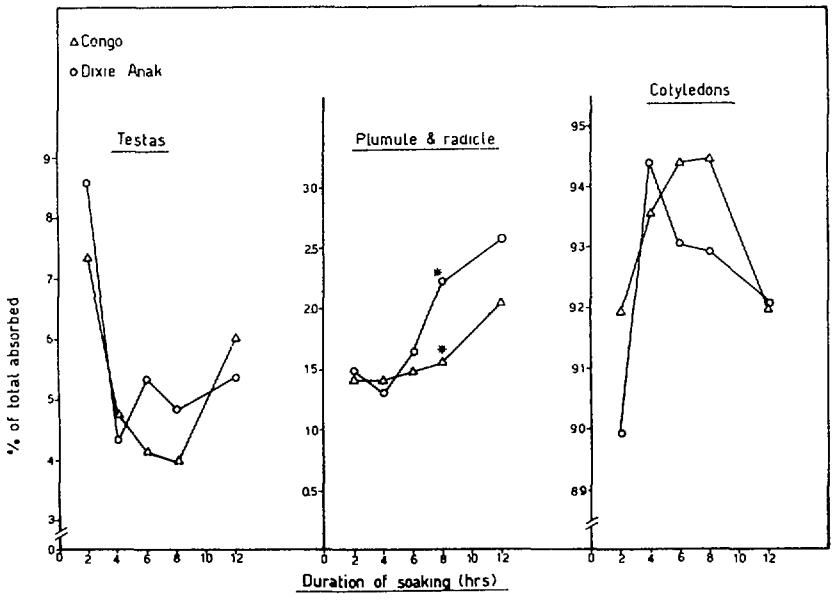

Fig. 3. The effect of the duration of soaking on the EMS distribution (%) in different parts of the seeds, from Brandstien, 1980.

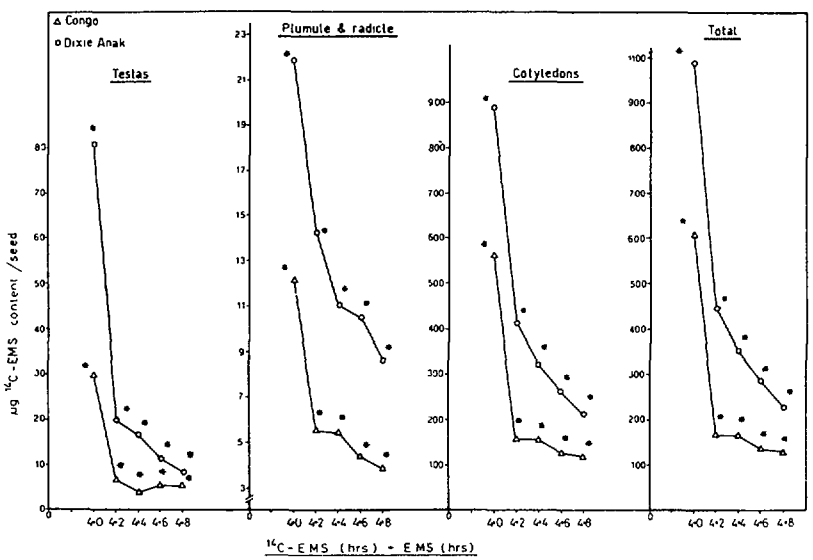

Fig. 4. The influence of the duration of soaking of the mutagen in cold EMS following 4 hr soaking in ^{14}C-labelled EMS, on the distribution of the label in the different parts of the seeds (μg); from Brandstien, 1980.

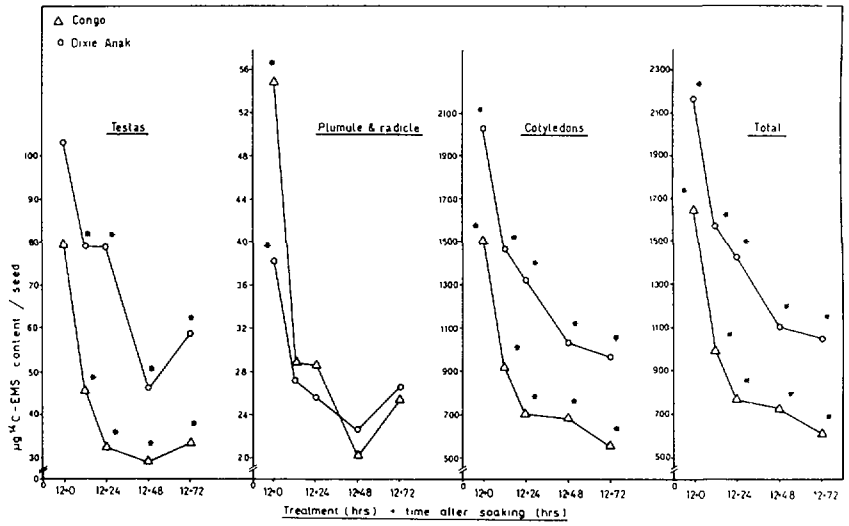

Fig. 5. The amount of ^{14}C-EMS (µg) in different parts of the seeds of two peanut cultivars, following 0-72 hr of germination in Petri dishes. (* denotes a significant difference, 5% level, between varieties); from Brandstien, 1980.

EMS was absorbed by the seeds than expected on the basis of the tritiated water uptake. Thus, it was confirmed, as shown also in herbicide studies, that the mutagen is taken up by diffusion. There was a difference in the amount of the mutagen taken up by seeds of the two cultivars - with higher amounts in the seeds of Dixie Anak, which was known to be more sensitive physiologically. The amounts of EMS-label in the different parts of the seeds are shown in Fig. 2. Their partitioning in the testa, cotyledons and plumule & radicle is shown in Fig. 3. Most of the ^{14}C label was absorbed by the cotyledons; very little was present in the plumule & radicle (ca. 2% in Congo and 2.5% of the amount in Dixie Anak; or 50 µg EMS/plumule & radicle/ seed in Congo and 65 µg in Dixie Anak). Thus, the amount of the mutagen reaching the meristematic tissue, target site for mutation induction, is very small. While at 12 hr of soaking the amount of label in the cotyledons seemed to reach a plateau, in the plumules & radicles it was still climbing and very small.

Pulse treatments in which soaking in labeled EMS was followed by soaking in unlabeled EMS showed that initially the level of the labeled mutagen dropped rapidly and then more slowly (Fig. 4). Similar results were obtained in germination trials (in Petri dishes) following EMS treatments (Fig. 5).

In view of these results it is recommended that very short treatments will be avoided when the testas delay the penetration of the mutagen. Pre-soaking in water which leads to testa splitting should be helpful. It is evident that very small amounts of the mutagen reach the meristematic tissue, the target cell initials, and that these amounts can be modified by several factors. Hence the calculation of mutation yields in relation to mutagen concentration in the solution should be made with caution.

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IMPROVEMENT OF MUNGBEAN BY X-RAY IRRADIATION*

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Abstract

In order to improve the yielding potential of mungbean in Korea and reduce crop losses due to shattering, diseases and pest a mutation breeding experiment is undertaken. The paper presents the results up to the M_3 generation.

Introduction

Although mungbean could contribute to increase the farm-income through an inter-cropping and could diversify the diet as a protein source, breeding work and systematic studies of the crop have not been carried out satisfactorily in Korea. At present, the major problems of mungbean production in Korea are the low yielding potential and the pod shattering which forces farmers to harvest individual pods as matured. To solve these problems, a large collections of germplasm was evaluated by our Laboratory. Some varieties were promising in yield and seed size, while no gene sources were found for non-shattering and for Cercospora disease and aphid resistance. Hence, our project considers to improve such main defects of this crop through induced mutations.

Our breeding aims, the scheme of research works, the materials adopted and the experiment with X-rays had already been described before (1) so that the results up to M_3 generation are presented and discussed in this paper.

1. Selection for desirable agronomic characters and progeny tests of selected mutant lines

1) M2 generation:

In order to select for desirable characters such as shattering resistance, Cercospora leaf spot resistance, seed yield and yield components from M_2 population of the varieties Kyunggi #5 and M-317, 20 healthy seeds from each M_1 plant were planted on May 18, 1979 (Table 1). A plot consisted of a single row of 2 m in length, 60 cm apart and spaced 10 cm between seeds. The controls (parent varieties) were planted in every 20th testing row. During the entire growing period, each plant within the lines was carefully evaluated in various agronomic characters.

Radiation effects on several important agronomic characters of the M_2 population, such as number of pods per plant, number of clusters per plant and number of seeds per pod were evaluated and the results are presented in Table 2, 3 and 4. There was significant variability for number of pods per plants and number of clusters per plant in the M_2 population as compared to the control, whereas the variability in number of seeds per pod was not considerable (2).

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A number of desirable mutants selected from M_2 populations of two mungbean varieties were summarized in Table 5. The selection was based on a visual examination of growing plants in the field. Of 423 selected mutants, 187 plants were from the M_2 population of Kyunggi #5 and 236 plants from the M_2 population of M-317. Out of the M_2 population of the variety M-317, 19 mutants intermediately resistant against Cercospora were selected. However, a mutant with aphid resistance was not found. Apart from the breeding aim, several mutant types such as unifoliata, multifoliata, sterile, partly fertile, and short podded were also found in the M_2 population (3, 4). In addition to the selected mutants, one healthy pod from each remnant M_2 plant was collected and mixed to prepare a M_3 bulk population.

2) M3 generation

A total of 404 mutant lines selected for shattering resistance or with other desirable agronomic traits were planted in single rows with two replications on 4th of June in 1980. Each row was 3 m in length with 50 cm between rows and 10 cm spacing within the rows. The mother varieties Kyunggi #5 and M-317 were planted in every 20th row as a control.

The 1980 growing season at KAERI Experiment Farm was below normal temperature and over rainfall with high humidity during July through September. It was unfavourable climate to evaluate the degree of shattering under field condition. Therefore, we laid emphasis on the evaluation of seed yield with several important agronomic characters of each M_3 mutant line. From the results of progeny test of selected mutant lines, the phenotypic variability for seed yield and other traits is summarized in Table 6. Characters measured on a plot basis were as follows: (1) seed yield harvested from a two meter section of each row (2) seed weight, recorded as grams per 1,000 random whole seeds (3) days to first flowering and ripe pod, recorded as the number of days from planting to the date when a plant in the plot had the first flower and ripe pod (4) aphid and mungbean mosaic virus, recorded from 1 to 5 where 1 indicates almost all plants resistant and 5 all plants damaged severely. Other characters were recorded as total means of three plants selected at random in each replication at maturity.

A wide range of variation was observed in some traits like seed yield, number of branches, number of pods, number of clusters, reaction to mungbean mosaic virus and aphid infestation. Other characters showed comparatively a lower range of phenotypic variation. Seed yield ranged from 695-2159 kg/ha with a mean of 1308 kg/ha in Kyunggi #5 and 296-1211 kg/ha with a mean of 696 kg/ha in M-317, respectively. The results show that the yield potential of the M_3 population of Kyunggi #5 is higher than that of M-317. With the comparison of their controls, high yielding mutant lines were selected from the population of two varieties (Table 7). Out of mutant progenies of Kyunggi #5, eight lines were selected as promising lines with high yield potential with increased seed size. Among them, the mutant line no. II-RS-38 showed superior yield potential (more than 2,000 kg/ha). From the progenies of M-317, five lines were selected as high yield mutants. The selected lines will be evaluated for their seed yield and other characters. In addition, one healthy plant from each M_3 line was harvested individually and will be grown as plant to row in M_4 to screen for shattering resistance.

Since heavy infection with mungbean mosaic virus (M.M.V.) and aphid infestation occurred in 1980, overall damages in M_3 generation were assessed and recorded on a scale of 1 (free) to 5 (severe) (Table 8). Out of 187 M_3 lines in variety Kyunggi #5, three lines scored 1-2 in M.M.V. and four lines scored 2 in aphid. The overall mean for M.M.V. and aphid was 3.7. Especially the line no. II-RS-13, scoring 1 seems to be promising. Out of 236 M_3 lines in variety M-317, 10 lines scored 2 regarding aphid damage but there was no resistant line to M.M.V. The overall means for the disease and insect scores were 4.3 and 3.3, respectively. In addition, a rigid selection for disease and insect resistance was also made on individual plant basis. The selected lines, in particular no. II-RS-13 and 50 plants will be evaluated for resistance to M.M.V. and aphid by both natural and artificial inoculation methods in 1981.

Meanwhile, the seed harvested from the 19 mutant plants intermediate resistant to Cercospora leaf spot (CLS) disease from M_2 population of variety M-317 in 1979 had been tested for CLS resistance under the natural field conditions. A part of the seed lots from the mutants was also planted in a greenhouse and inoculated artificially by spraying a spore suspension with 0.1 percent of Tween 20 at 3-4 leaf stage of growth. The inoculated seedlings were covered with polyethylene film and misted for a day to keep the humidity favourable for infection. We could not find any resistant mutant to CLS disease in the material tested by artificial inoculation.

2. Preparation of M_1 population by thermal neutron treatment

The shattering of pod is one of the limiting factors in mungbean production in Korea. However, a shattering resistant gene source is not available in the collection we have made in the past years.

Based upon our experience in induction of shattering resistant mutants by thermal neutron irradiation in soybean (5), two M_1 mungbean populations were prepared by thermal neutron irradiation with the varieties, Kyunggi #5 and M-317. The seeds containing 12 percent in moisture were exposed (100 seeds/dose) to various doses of thermal neutrons as 10, 20, 30, 40, 50 and 60 x 10^{12} $th_N/cm^2/sec$ in the thermal column of TRIGA MARK-II reactor at KAERI. All treated seeds were immediately planted in a sand bed of a greenhouse with three replicates. 15 days after emergence, germination rate, seedling height and root length were measured to determine radiosensitivity and optimum dosage for practical seed treatment. The effects of thermal neutrons on germination, seedling height and root length are presented in Fig. 1. A gradual decrease in seed germination, seedling height and root length with increased doses was observed under greenhouse condition. Reduction in seedling height and root length at high doses occurred more in Kyunggi #5 than in M-317.

With reference to the results obtained, 3,000 dry seeds per dose from each variety were irradiated with 20 and 30 x 10^{12} $th_N/cm^2/sec$ of thermal neutrons. The treated seeds have been grown in the field together with the mother varieties in rows spaced 50 cm between rows and 5 cm between plants. After emergence, drastic plant killing occurred mainly during the first four weeks of growth. The treatment of 30 x $10^{12}/cm^2$ around 23 percent seedlings survived in 66 days from planting. The number of plants harvested is presented in Table 9. We could only get M_2 seeds from 993 M_1 plants of Kyunggi #5 and 671 plants of M-317, because of low temperature during September 1980. The M_2 seeds harvested from the M_1 generation will be planted for screening the resistance to shattering in the middle of June 1981.

3. Estimation of yield loss by Cercospora leaf spot

Despite the fact that a considerable yield reduction is caused by Cercospora leaf spot (CLS) in mungbean in Korea a accurate estimation of yield loss has not been made yet. Therefore, an experiment was designed to assess the effect of CLS epidemics on mungbean yield with different planting dates.

Two mungbean varieties, Kyunggi #5 and M-317, were planted in the field in rows of 3 m long with 60 cm between rows and 10 cm between seeds at three planting dates, June 16, July 2 and July 18. The split plot design with three replicates was used for this experiment. Treatments were (1) check (2) inoculation, and (3) control sprayed with 0.1% Benlate. The control plots were sprayed to run-off with 0.1% Benlate forthnightly, from seedling emergence until maturing. The inoculation plots were sprayed with an aqueous spore suspension containing 2×10^3 spores/ml and 0.1% Tween 20 by air compressor to wet thoroughly leaf surface at 4-5 leaf stage of mungbean. Yield data were taken from bottom, middle and top parts of the plants in a centre row of each plot after the plants 50 cm from the ends were removed at harvest. Disease severity was measured by the portion of diseased leaf area a month after inoculation with a modified disease scoring system proposed by Schneider et al. (6).

Total yield was greater in planting date July 2 than June 16 in the variety Kyunggi #5, but the reverse was true for th variety M-317. This seemed to be due to late maturity of the variety M-317. Yields were significantly reduced by CLS infection, 11.4 - 21.4% in inoculated plots and 5.1 - 18.2% in check plots depending upon mungbean varieties and planting dates as compared to controls sprayed with fungicide (Table 10). The analysis of variance showed significant difference in yield of the variety Kyunggi #5 among planting dates and treatments caused by the disease (Table 11). Yield reduction by the disease was more significant in the middle and top parts of the plants as compared to that of bottom parts. This tendency also appeared in the variety M-317, although there was no statistical differences among the treatments (Table 12). Negative correlation of yield with percent of diseased leaf area was found. Regression co-efficient values were a little lower in planting date, June 16 and July 2 in both varieties (Fig. 2). Seed weight was significantly affected by planting date and CLS infection, especially in the upper part (Table 13). Other agronomic characters, except numbers of pods per plant, seemed to be affected by treatments (Tables 14 & 15). Yield loss by CLS infection occurred primarily through the reduction in seed weight (seed size) and number of pods per plant as one would expect with a late season foliage disease.

Results confirmed that yield reduction by CLS epidemics was considerable and significantly correlated with the disease severity. The relationship between the severity of the disease and yield reduction would be expressed by the regression of yield reduction on the disease severity at reproductive stage of mungbean. The combined data of both cultivars were used to calculate linear regression equations for the estimation of yield reduction based on the disease severity: $Y = 6.24 + 0.16 x$ in which Y = predicted percent yield reduction, X = CLS severity at reproductive stage, $r = 0.583^{**}$ (d.f. = 28) (Fig. 3). However, further study is needed to test and improve this equation for the estimation of yield reduction by CLS epidemics.

4. Preliminary study to a screening technique for Cercospora leaf spot resistance at early growth stage

Cercospora leaf spot (CLS) caused by Cercospora canescens appears severe in late stage of the growth, but under ideal environmental condition, heavy infections of the CLS may reduce yields as much 37 percent (7).

According to the report of Mew et al. (8) susceptible varieties showed no differences in response with age up to flowering, but the severity increased drastically from the flowering stage onward when they were artificially inoculated with CLS. This indicates that screening for

resistance to CLS in the early growth stages is likely to be difficult under field conditions. On this account, it is imperative to develop a useful screening technique for resistance to CLS.

1) Sporulation

Few attempts to induce sporulation of Cercospora spp. in vitro have been successful. Workers (9, 10) have subjected isolates to manipulation of nutrition, light and temperature and concluded that the erratic sporulation of Cercospora spp. in culture partially was due to the nature of the medium and partially to other factors. Hence, this study of in vitro production of conidia by CLS fungus was conducted to obtain a supply of conidia sufficient for screening mungbean varieties and mutant lines for a source of resistance.

The isolates of Cercospora canescens were obtained from spores picked by single spore isolation method from infected mungbean leaves that had been kept in a moisture chamber 3-4 days at 25-27°C. Spores were placed on potato dextrose agar media (PDA) and incubated at 27°C for two weeks. Various media were evaluated for ability to support sporulation. Several standard media, PDA, oatmeal agar (OA) and mungbean leaf decoction oatmeal agar (MOA) were tested. Cultures were incubated under various lighting conditions to determine whether light effected sporulation (Table 16). Spore and mycelial suspension was flooded on the media and inoculated at 28°C in the dark under fluorescent light (approximately 2,500 Lux) and alternate lighting for 8 hours and dark for 16 hours. Petridishes of culture plates were uncovered for illumination because glass lids would filture out ultraviolet radiation. Inoculum virulence was tested by inoculating mungbean plants of 4-5 trifoliolate leaf stage grown in pots. Spore suspension mixed with 0.1% Tween 20 were atomized onto mungbean leaves to run off. Inoculatd plants were placed in a mist chamber for two days at 25-30°C and then placed on a greenhouse's benche to allow symptom development.

Isolates of Cercospora canescens cultured on PDA showed only a typical dense mat of mycelium with a reddish purple pigment in the medium surrounding the colony. Vegetative growth of the fungus occurred in the medium with no significant difference between the media, but there was a tendency of slight increase in PDA and higher temperature (Fig. 4). Abundant sporulation occurred in culture on MOA exposed to about 2,500 Lux of fluorescent light, but it did not occur in continuous darkness (Table 16). The conditions that produced maximum number of conidia did not coincide with those for vegetative growth and pigmentation in culture medium. Removal of aerial mycelium in culture by brushing with sterile water enhanced the conidial production so that OA could be useful for production of abundant conidia (Table 17 & Fig. 5). Three weeks after inoculation of mungbean plants with spore suspension prepared from two different media, brown lesions with necrotic center developed on the lower leaves (Table 18). This suggests that the OA can be used for sporulation of Cercospora canescens without changing virulence.

2) Screening technique

It was tried to use culture filtrates of CLS for the screening. CLS was cultured in Frei's liquid medium (11) for 60 days at about 25°C and culture filtrates of CLS

were prepaed by filtration of the liquid culture through Whatman filter paper no. 3. For a determination of growth inhibiting activity of the filtrates, mungbean seeds were treated to be germinated in different concentration of the filtrates and on germination, hypocotyl elongation was measured by weighing of dry matter. In this treatment, a marked inhibition was found in growth of stem and hypocotyl, even in 10^{-4} dilution of the filtrates (Fig. 6), whereas the germination percent was not influenced (Table 19). If the culture filtrate of CLS has a host-specificity, it is likely to be applicable in screening for CLS resistance in early growth stage.

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Table 1. Number of M_2 lines and plants grown in 1979.

Variety	Dosage	No. of M_2 lines	No. of seeds /row	No. of M_2 plants
Kyunggi #5	X-ray 30kR	1,950	20	39,000
	40kR	1,770	20	35,400
				74,400
M-317	X-ray 30kR	1,167	20	23,340
	40kR	990	20	19,800
				43,140
Total		5,877		117,540

Table 2. Variations for number of pods/plant in the M_2 and control populations of mungbean varieties, Kyunggi #5 and M-317.

Variety	No. of plants examined	No. of pods per plant															Max.	Min.
		0-5	5-10	10-15	15-20	20-25	25-30	30-35	35-40	40-45	45-50	50-55	55-60	60-65	65-70	>70		
Kyunggi #5																		
M_2 population	905		13	53	119	144	187	125	97	71	34	30	17	7	3	5	94	5
Control	80			1	8	16	13	13	13	7	5	2	2				56	13
M-317																		
M_2 population	916		103	313	260	144	64	20	9	2	1						45	5
Control	80	2	15	26	28	5	4										29	4

Table 3. Variations for number of clusters per plant in the M_2 and control populations of mungbean varieties, Kyunggi #5 and M-317.

	No. of plants examined	No. of clusters per plant															Max.	Min.
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15		
Kyunggi #5																		
M_2 population	905	1	31	184	233	170	123	63	48	29	16	4	2	1			13	1
Control	80		2	19	24	13	7	7	3	2	2	1					11	2
M-317																		
M_2 population	916		31	119	134	169	157	106	86	51	23	20	8	7	3	2	16	2
Control	80		4	17	23	20	8	6	2								8	2

Table 4. Variations for number of seeds/pod in the M_2 and control populations of mungbean varieties, Kyunggi #5 and M-317.

Variety		No. of plants examined	No. of seeds per pod										Maximum	Minimum
			7	8	9	10	11	12	13	14	15	16		
Kyunggi #5	M_2 population	905	2	2	8	32	94	205	265	217	77	3	16	7
	Control	80			4	8	10	12	17	24	5		15	9
M-317	M_2 population	916		1	7	25	48	144	264	282	134	11	16	8
	Control	80			1	1	3	9	15	27	20	4	16	9

Table 5 . Mutants selected in the M_2 populations in two mungbean varieties.

Material	Shattering resistance	Heavy podding	Early maturing	Vigorous plant	Resistant to CLS	Long pod	Total
Kyunggi #5	116	52	4	4	0	11	187
M-317	121	60	23	13	19	0	236
Total	237	112	27	17	19	11	423

Table 6. Variability for seed yield and important agronomic traits of M_3 mungbean populations grown in 1980.

	Maximum		Minimum		Average		S.D.*		C.V.(%)	
	K-5**	M-317	K-5	M-317	K-5	M-317	K-5	M-317	K-5	M-317
Yield (kg/ha)	2159.0	1211.0	694.7	296.4	1308.4	695.5	610.60	175.26	46.67	25.20
Seed wt. (gr/1000)	48.8	61.6	34.1	36.8	41.5	53.9	2.86	3.03	6.89	5.62
Plant ht. (cm)	67.6	70.8	39.1	44.2	53.2	60.1	3.37	4.90	6.33	8.15
No. of pods/plt.	50	23	13	10	25.3	14.7	5.62	2.50	22.22	17.00
No. of clusters/plt.	8	10	3	2	4.7	5.9	0.78	1.49	16.60	25.24
No. of branches/plt.	3	4	1	0	1.7	1.6	0.67	0.61	39.41	38.13
No. of nodes/plt.	9	11	6	8	7.7	9.6	0.69	0.91	8.96	9.50
No. of seeds/pod	15	16	10	10	13.0	13.3	0.97	0.99	1.81	7.47
Pod length (cm)	9.8	14.6	7.6	7.6	8.9	9.9	0.54	0.73	6.08	7.35
Days to 1st flower	61	74	49	54	53.4	61.6	2.62	3.79	4.91	6.15
Days to 1st ripe pod	87	96	72	74	77.5	86.1	3.06	9.31	3.95	10.82
Aphid (1-5)	5	5	2	2	3.7	3.4	0.71	0.66	19.30	19.54
M.M.V. (1-5)	5	5	2	2	3.6	4.3	0.62	0.84	17.19	19.77

* S.D. : standard deviation, ** K-5 : var. Kyunggi #5

Table 7. High yielding mutant lines selected from M_3 population treated with X-ray in mungbean.

Entry no.*	Yield (kg/ha)	Seed wt. (g/1000)	Plant ht. (cm)	No. of pods /plant	1st ripe pod (days)	Aphid (1-5)	M.M.V. (1-5)
			var. Kyunggi #5				
RS-38	2159.0	44.1	54.0	39	77	3	3
IP-4	1999.1	45.3	56.2	25	73	4	2
RS-28	1915.5	41.4	56.2	28	76	3	4
IP-50	1821.1	42.3	51.8	26	77	4	3
RS-31	1788.2	45.9	62.0	27	76	3	3
IP-5	1751.1	45.6	54.4	22	73	4	3
RS-40	1712.2	44.9	55.0	30	77	3	3
IP-11	1677.3	45.2	54.4	23	74	3	4
Control	1471.3	39.8	50.2	26	76	4	4
			var. M-317				
RS-14	1211.0	53.3	56.5	14	86	3	4
RS-17	1170.8	55.2	65.4	19	86	3	4
RS-92	1156.6	52.0	58.6	16	81	4	4
RS-100	1140.2	58.4	63.5	14	83	3	4
LP-4	1127.7	54.1	60.0	14	87	3	3
Control	899.0	54.1	54.4	16	85	3	4

* RS = resistant to shattering, IP = increasing pod no., LP = long pod

Table 8. Frequency of score of aphid and mungbean mosaic virus (M.M.V.) in M_3 generation of two mungbean varieties.

Variety	No. of lines observed	Disease & insect	Score* 1	2	3	4	5
Kyunggi #5	187	Aphid	0	4	63	96	24
		M.M.V.	1	2	76	88	20
M-317	236	Aphid	0	10	121	86	9
		M.M.V.	0	0	24	125	87

* Score of 1 is highly resistant and 5 is highly susceptible.

Table 9. Preparation of M_1 population by thermal neutron treatment.

Variety	Dosage ($\times 10^{12}$ th_N/cm^2)	No. of seeds sown	No. of plants harvested
Kyunggi #5	20	3,000	701
	30	3,000	292
	Total	6,000	993
M-317	20	3,000	510
	30	3,000	161
	Total	6,000	671

Table 10. Effect of Cercospora leaf spot epidemics on mungbean yield at different planting date.

Variety	Treatment	June 16					July 2					July 18				
		B*	M	T	Total	Reduction (%)	B	M	T	Total	Reduction (%)	B	M	T	Total	Reduction (%)
Kyunggi # 5	Inoculation	591.7	479.5	524.2	1595.4	18.5	590.9	776.1	450.9	1817.9	21.4	150.8	426.2	244.9	821.9	17.6
	Check	726.0	555.0	578.1	1859.1	5.1	578.1	787.7	524.3	1890.1	18.2	192.2	423.7	242.4	858.3	14.0
	Spray**	724.5	633.3	600.3	1958.1		830.9	879.1	601.5	2311.5		193.0	465.1	339.9	998.0	
M-317	Inoculation	567.0	504.2	555.0	1626.2	15.6	480.6	512.5	443.7	1436.8	11.4	-	-	-	-	
	Check	583.1	527.8	577.6	1688.5	12.3	508.4	542.3	459.8	1510.5	6.9	-	-	-	-	
	Spray	698.0	617.0	611.4	1926.4		522.6	625.9	473.2	1621.7		-	-	-	-	

* B, M & T : bottom, middle and top positions of the plants, respectively.

** Benlate was sprayed twice per month from primary leaf stage to maturing.

Table 11. Analysis of variance of seed yield in mungbean variety Kyunggi #5.

Source	d.f.	Seed yield (kg/ha)		
		Bottom	Middle	Top
Block (B)	2	16839.246*	66491.515	457.238
Planting date (P)	2	735589.029**	332901.043*	219151.029**
Error (a)	4	2285.478	39791.941	8914.922
Treatment (T)	2	43696.080*	23179.075**	26107.105**
P x T	4	18350.826	2856.730	1969.743
Error (b)	12	9416.344	2979.890	3019.883

*, ** : Significant at 5 and 1 percent level, respectively.

Table 12. Analysis of variance of seed yield in mungbean variety M-317.

Source	d.f.	Seed yield (kg/ha)		
		Bottom	Middle	Top
Block (B)	2	25649.280	26390.517	8978.851
Planting date (P)	1	56604.904	501.389	67479.134
Error (a)	2	26402.511	27595.441	7978.674
Treatment (T)	2	12135.788	20951.504	2773.867
P x T	2	4555.340	17.037	296.761
Error (b)	8	7760.040	10649.700	765.965

Table 13. Analysis of variance of seed weight in two mungbean varieties, Kyunggi #5 and M-317.

Source	d.f.	Var. Kyunggi #5			Var. M-317		
		B	M	T	B	M	T
Block (B)	2	0.014	0.025	0.018	0.007	0.069	0.007
Planting date (P)	2 (1)[a/]	0.272	0.188**	0.034	1.869	0.605	1.681*
Error (a)	4 (2)	0.027	0.005	0.039	0.009	0.027	0.071
Treatment (T)	2	0.014	0.023*	0.067*	0.029	0.029	0.061**
P x T	4 (2)	0.045	0.002	0.015	0.029	0.020	0.057**
Error (b)	12 (8)	0.022	0.005	0.012	0.017	0.032	0.005

a/ Parenthesis show degree of freedom for var. M-317.

*, ** : Significant at 5 and 1 percent level, respectively.

Table 14. Analysis of variance of important agronomic characters in mungbean variety Kyunggi #5.

Source	d.f.	Plant ht. (cm)	No. of branches /plt.	No. of nodes /plt.	No. of clusters /plt.	No. of pods /plt.	Pod length (cm)	No. of seeds /pod
Block (B)	2	52.181	0.482	0.778	0.333	13.482	0.029	0.037
Planting date (P)	2	172.408	10.815**	29.78 **	37.000**	1083.370**	1.449*	1.815
Error (a)	4	64.821	0.370	0.222	0.667	23.926	0.141	0.426
Treatment (T)	2	30.263	1.815	0.111	1.333	120.482**	0.000	0.037
P x T	4	5.564	1.037	0.389	2.167	36.759	0.062	0.259
Error (b)	12	11.970	0.574	0.407	1.111	11.944	0.067	0.574

*, ** : Significant at 5 and 1 percent level, respectively.

Table 15. Analysis of variance of important agronomic characters in mungbean variety M-317.

Source	d.f.	Plant ht. (cm)	No. of branches /plt.	No. of nodes /plt.	No. of clusters /plt.	No. of pods /plt.	No. of seeds /pod	Pod length (cm)
Block (B)	2	7.116	0.593	1.037	0.259	11.148	1.926	0.067
Planting date (P)	2	668.499*	9.593**	21.926*	53.482**	417.148**	0.704	0.752
Error (a)	4	40.887	0.315	2.037	1.537	8.370	3.315	0.629
Treatment (T)	2	31.434	0.037	0.148	0.259	9.593*	0.259	0.329
P x T	4	38.023	0.093	1.482**	0.370	1.482	0.907	0.229
Error (b)	12	16.671	0.796	0.204	0.333	2.352	0.907	0.094

*, ** : Significant at 5 and 1 percent level, respectively.

Table 16. The effects of light and medium on sporulation of Cercospora canescens.

Media	Light*	No. conidia $10^3/cm^2$ after indicated days			
		4	5	6	7
Potato	L	trace	0.3	0.3	0.9
dextrose agar	D	trace	0.3	0.3	0.6
	A	0.9	0.5	0.5	0.9
Oatmeal	L	3.2	18.2	36.1	48.2
agar	D	0.3	0.8	4.3	4.5
	A	5.5	15.8	37.0	48.4
Mungbean leaf	L	17.4	67.4	117.7	121.7
decoction agar	D	1.5	2.0	12.3	16.9
	A	14.8	65.1	117.4	120.5

* L = fluorescent light, D - dark, A = alternate light for 8 hrs and dark for 16 hrs.

Table 17. The effects of brushing culture on sporulation of Cercospora canescens.

Media	Light*	No. conidia $10^3/cm^2$ after indicated days			
		4	5	6	7
Potato dextrose agar	L	1.5	2.3	5.5	8.1
	D	trace	0.3	0.3	0.3
	A	1.7	2.3	7.3	8.4
Oatmeal agar	L	2.9	14.8	77.2	121.3
	D	trace	0.6	0.9	21.2
	A	3.8	16.0	77.2	122.0
Mungbean leaf decoction agar	L	2.6	26.8	81.2	179.2
	D	trace	2.9	3.9	21.2
	A	3.5	27.7	84.8	173.2

* L = fluorescent light, D = dark, A = alternate light for 8 hrs and dark for 16 hrs.

Table 18. Infection of mungbean plants inoculated with spores of Cercospora canescens formed on two different media.

Media	2 weeks		3 weeks	
	Brown spot	Necrotic spot(%)	Brown spot	Necrotic spot(%)
O A	247*	2.7	268	67.2
MOA	259	2.4	272	68.7

* Number of lesions were counted from five leaves beared on main stem at flowering stage.

Table 19. The effect of culture filtrate of Cercospora canescens to shoot growth of Mungbean.

Day \ Dilution	10^{0}	10^{-1}	10^{-2}	10^{-3}	10^{-4}	D.W.
			Germination(%)			
1	82.6	84.4	86.1	86.7	90.6	90.6
5	93.3	93.3	93.9	93.9	93.9	93.9
			Dry weight (mg)			
4	5.916	6.309	7.039	7.150	7.174	9.086
5	9.061	11.152	11.186	11.561	12.414	13.682

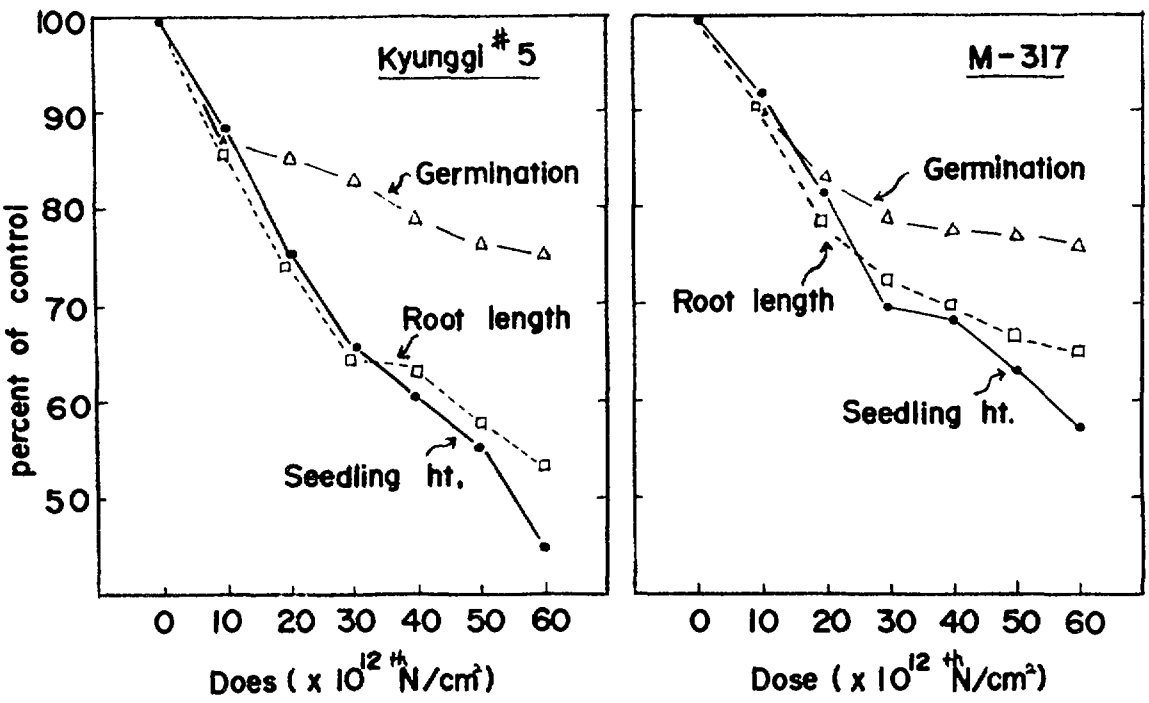

Fig. 1. Effect of thermal neutron on germination rate, seedling height and root length in two mungbean varieties under the greenhouse condition.

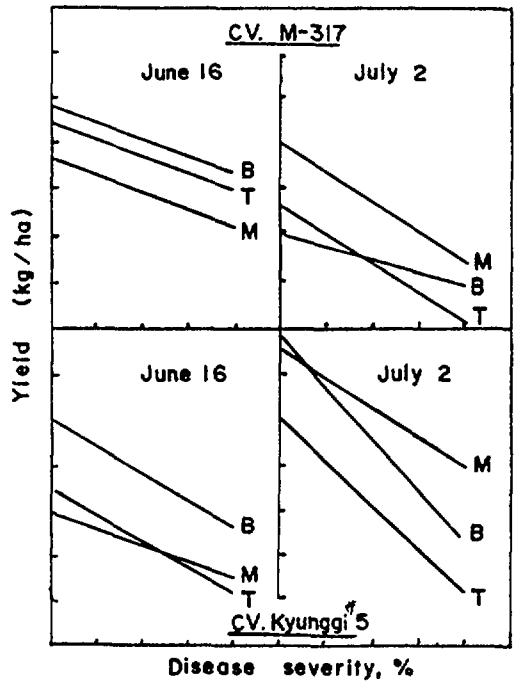

Fig.2.

Regression analysis of cercospora leaf spot severity and yields of mungbean varieties with different planting dates.

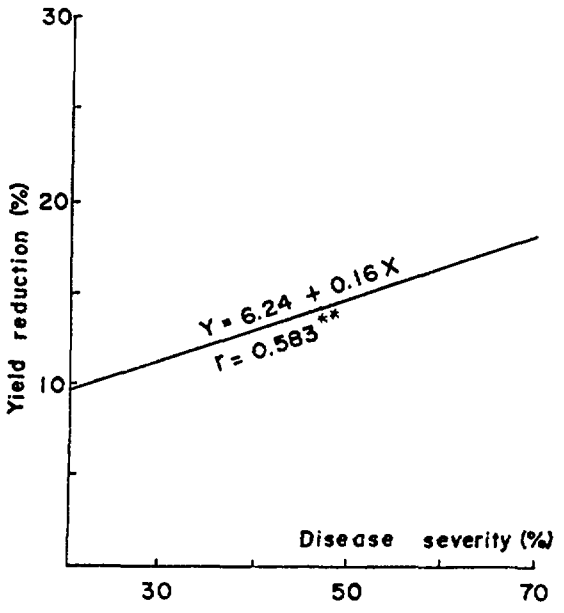

Fig.3

Relationships of cercospora leaf spot severity to percent yield reduction in mungbean.

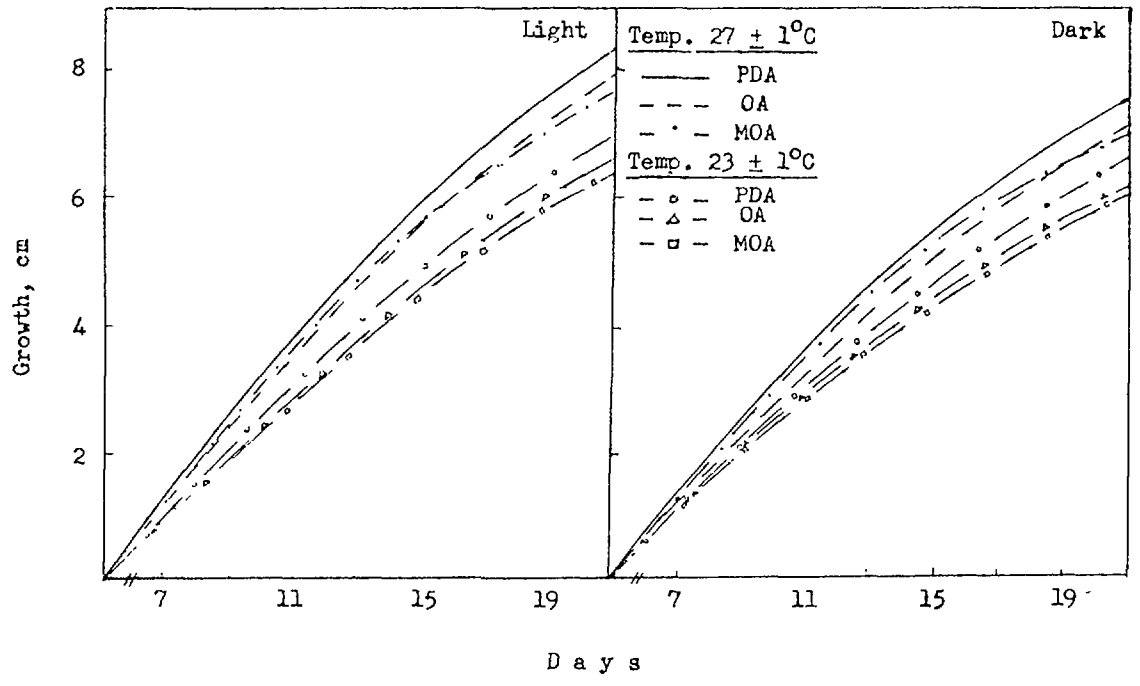

Fig. 4. Growth of Cercospora canescens on culture media under different conditions.

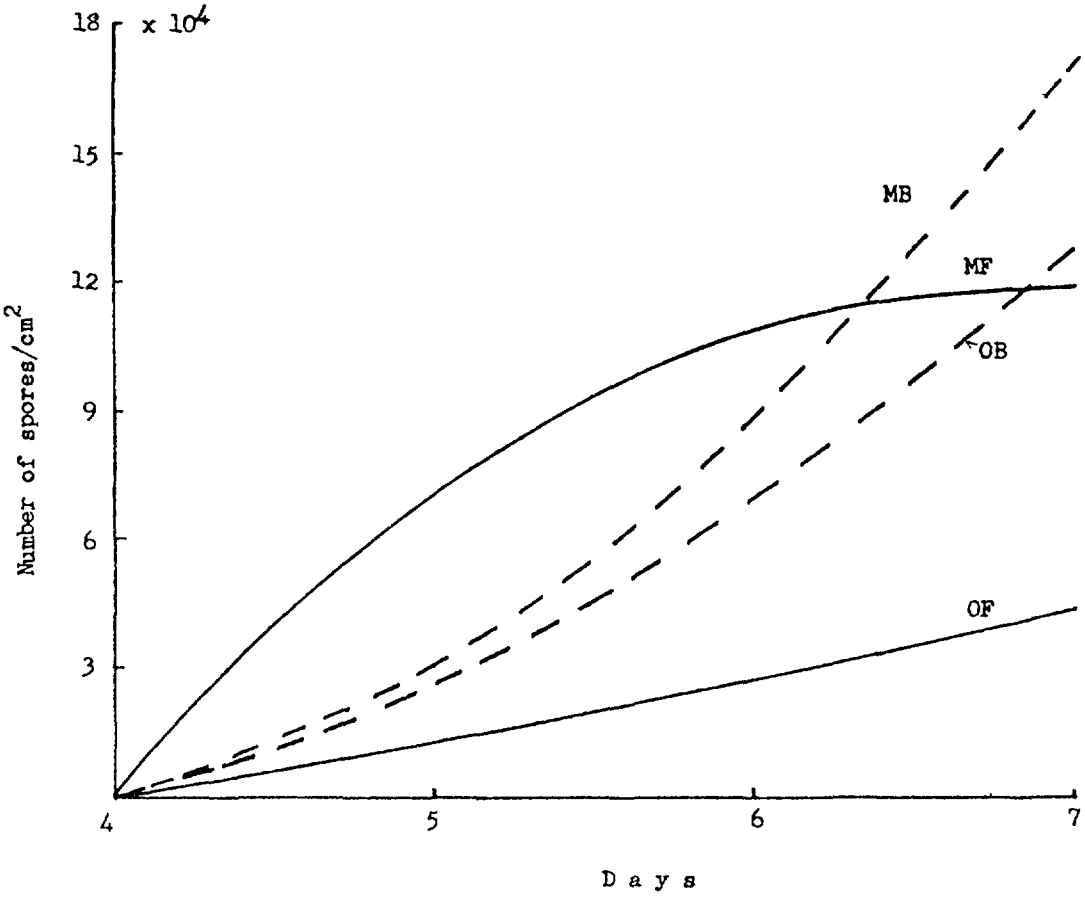

Fig. 5. Sporulation of Cercospora canescens on oatmeal agar (OF) and mungbean leaf decoction agar (MF) inoculated by flooding spore suspension, and the former (OB) and the latter (MB) brushed by sterile water three days after flooding.

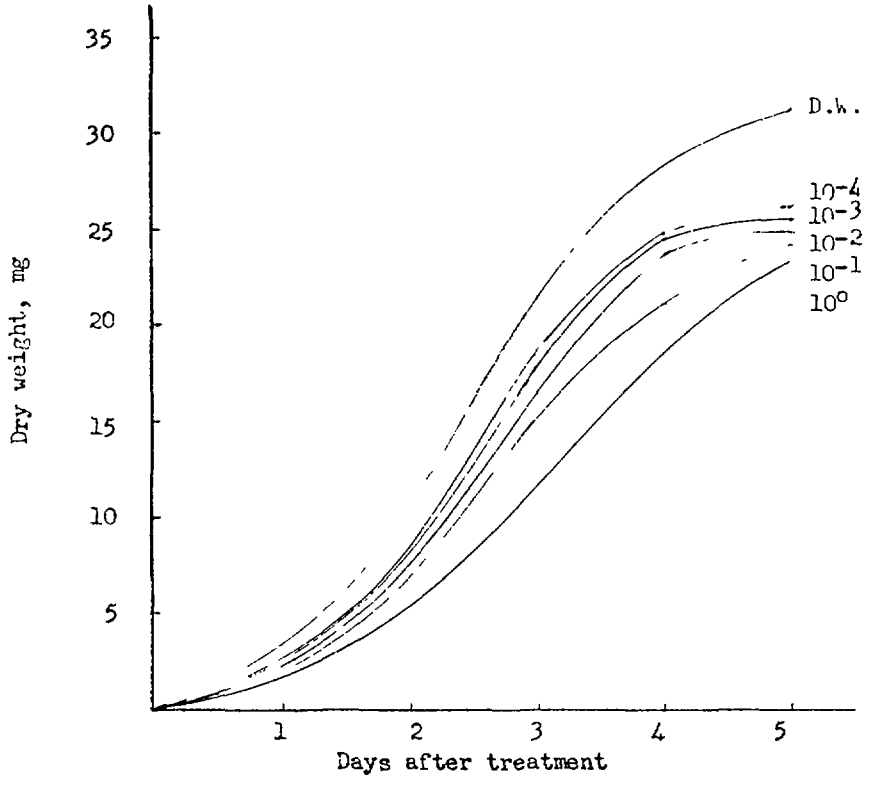

Fig.6. The effect of culture filterate of Cercospora canescens to hypocotyl elongation of mungbean.

MUTAGENIC EFFICIENCY OF ETHYLMETHANE SULPHONATE (EMS) IN SOYBEAN*

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ABSTRACT

To obtain the maximum advantage of using a chemical mutagen a high efficiency of mutant production must be aimed at. Efficiency is defined as the ratio of mutations/biological damage. The mutations considered are the M_2 chlorophyll mutants while the criteria for measuring the biological damage include M_1 parameters such as reduction in seedling height, reduction in survival, and sterility.

This paper reports the effects of presoaking and postwashing on the efficiency of EMS in soybean. Seeds of variety Palmetto were presoaked for varying periods of 0, 6, 12 and 24 hours, treated with 0.2% EMS for 3 hours and postwashed in different lengths of time of 0, 6, 12 and 24 hours.

Efficiency appears to be enhanced with increasing periods of presoaking. This is evident when plant injury and sterility are taken as the criteria for biological damage. However when lethality is considered, a presoaking period is not necessary for efficiency to be increased. The results on the effects of postwashing seem to point to an optimum period of 12 hours for postwashing. Efficiency in relation to injury, lethality and sterility tends to decline when postwashing is prolonged to 24 hours.

INTRODUCTION

Ethyl methanesulphonate (EMS) is one of the most widely used chemical mutagens in crop improvement today. When compared to ionizing radiations it induces more viable mutations (Gaul, 1964), even though most of the macromutants induced are accompanied by sterility. A substantial amount of literature on the methods of mutagen treatment using EMS has been accumulated but almost all of it is in cereals, notably barley (Konzak et al., 1972). Very little has been published on the methodology of chemical mutagenesis in soybean besides some preliminary studies on dose response (Constantin et al., 1976).

To obtain the maximum advantage of using a chemical mutagen a high efficiency of mutant production must be aimed at. "Efficiency" as used in this report was defined by Konzak et al., 1965, as the ratio of "factor mutations/biological damage". The objective is a high efficiency by having a low biological damage and yet a high frequency of mutations.

Many variables are involved in the interaction between the chemical mutagen and the cells of the embryo. To obtain reproducibility the experimenter must be able to control these variables. Two important variables that should receive attention are the presoaking period and the duration of postwashing after seed treatment. Presoaking of seeds in water permits better control of the rate of penetration of the mutagen while postwashing enables the elution of the remaining mutagen in the seed tissues and the hydrolytic products which may be toxic (Konzak et al., 1972).

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This paper reports the investigation on the effects of presoaking and postwashing on soybean seeds. The criteria for measuring the biological damage include reduction in seedling height (plant growth injury), reduction in survival (lethality) and sterility in M_1 plants. The factor mutations scored are those of chlorophyll mutants in M_2 generation.

MATERIALS AND METHODS

The soybean variety Palmetto was used as experimental material.

Presoaking experiments

Seeds were placed in nylon mesh bags and presoaked for various lengths of time (0, 6, 12 and 24 hours) at room temperature (26°C) and agitated from time to time to allow free movement of water. After presoaking, seeds were treated with 0.2% EMS for three hours at 26°C. Finally the seeds were postwashed for a period of 6 hours under running tap water at the rate of 4 litre/minute.

Postwashing experiments

Seeds of soybean which had been presoaked for 6 hours were treated with 0.2% EMS for three hours at 26°C and then postwashed for varying periods of 0, 6, 12 and 24 hours under running tap water at the rate of 4 litres/minute.

Greenhouse planting

All treatments were carried out in 3 replications. The seedling injury test consisted of 20 seeds per treatment planted at random in wooden boxes in the greenhouse. Measurement was taken when the first pair of leaves had stopped growing, around 14 days after emergence.

Dry weight of seedlings per treatment was obtained by drying samples at 110°C for 24 hours.

For the field planting, 100 seeds per treatment replicated 3 times were planted at random in 50-cm rows with 10 cm between plants. The rate of survival was determined at the time of flowering, and survivors were defined as those plants that produce at least one inflorescence regardless of whether seeds were going to be produced (Gaul, 1977). Seed sterility was examined at harvest time by taking 10 representative plants at random from each replicate and taking the ratio of missing seeds to total seeds produced. Seeds harvested during the M_1 were bulked per treatment and planted for the M_2. Seedlings were scored for chlorophyll mutants, classified and recorded by a modification of the system proposed by Gustafsson (1940). The types scored were albina, alboviridis, viridis, xantha, tigrina and maculata.

Measurement of mutagen outflow from embryos

The procedure described under postwashing experiment was repeated, but 14C-Methylmethane sulphonate (MMS) was used instead of EMS. 0.1 ml non-active MMS and 1 ml absolute ethanol were added to C-MMS to make up a stock solution with a dose of 50 μCi. To each treatment was added 1.1 μl of stock solution with an activity of 14C 1.11×10^5 dpm. 25 seeds from each treatment replicated three times were presoaked for 6 hours, incubated in the MMS solution for 3 hours and postwashed as specified earlier. The embryos were dissected and homogenized in 5 ml absolute ethanol and centrifuged for 10 min. at 10 revs/min. 10 ml of toluene - PPO-POPOP solution was added to the supernatant and the samples were counted in a liquid scintillation counter. The method was modified from Mikaelsen et al. (1968).

RESULTS

Table 1 presents the influence of presoaking on the EMS effects upon the M_1 generation. The control consisted of dry seeds without

any treatment. The seedling height was slightly more decreased with no presoaking. Field germination was more suppressed with presoaking. The seedling dry weight was lowest without presoaking. Survival appeared not to be affected by variation in presoaking. The lower survival rate following 12 hours presoaking was related to the low percentage of germination. Fertility seemed to slightly increase with increasing period. The maximum frequency of chlorophyll mutations in M_2 is obtained without presoaking (Table 2).

The efficiency of mutagen treatment as influenced by presoaking is assessed in Table 2. Admittedly the size of the M_2 population which the mutant frequency is based on is small and caution must be exercised in interpreting the results. The 12 hours presoak treatment was left out due to lack of data. Since the treatment had no effect on lethality (Table 1) this parameter could not be used to calculate efficiency. There appears to be no clear pattern for the effect of presoaking on seedling injury and sterility. When seeding injury and sterility are taken as a base efficiency tends to be enhanced with increased presoaking.

The influence of postwashing on EMS effects is outlined in Table 3. There is a general assumption that some postwashing is needed, but a prolonged time may be harmful. Without postwashing the germination percentage (75.3%) was lower, but it seems to improve with increasing postwash period. The seedling height, seedling dry weight and fertility also appear to improve when postwashing is carried out. For survival, the optimum is 6-12 hours of postwashing. The maximum frequency of M_2 chlorophyll mutants is obtained after 12 hours postwashing (Table 4).

A comparison on the efficiency of the various postwash periods is presented in Table 4. A 12 hour postwash is optimal for the efficiency of mutagen treatment.

Figures 1 and 2 show the comparative response of two varieties of soybean, Palmetto (mean 100-seed weight 11.60 *g*) and Tai-Ta Kaisung (mean 100-seed weight 14.50 *g*) to the effects of presoaking and postwashing. Figure 1 shows the response of seedling height and seed set to varying presoaking times. In figure 2 the effects of postwashing on seedling height and seed set are demonstrated. There are only minor differences between the two varieties. A period of 6-12 hours seems optimal for presoaking as well as postwashing. Figure 3 depicts the fate of a mutagen during different hours of postwashing. It is evident that after 12 hours of postwashing the elution of a mutagen from the seed tissues reached a plateau.

DISCUSSION

By presoaking the seeds the hydration of their cells is increased and this is supposed to result in a faster diffusion of EMS into the embryo of barley (Walles, 1967). The results obtained in this study with soybeans show that presoaking does not enhance the mutation rate and efficiency of mutagen treatment very much. Although there was a positive effect at the 24 hour period of presoaking, the frequency of M_2 chlorophyll mutants was not increased. One would suspect that the optimal time to be less than 24 hours, based on figure 1 where it seems that the period around 6-12 hours of presoaking had the desired influence on seedling height and seed set. More work is needed to establish reliable guidelines.

Postwashing has two advantages. In the first instance non-reacted EMS and the hydrolytic products could be removed from the seeds. This would define more precisely the treatment time and increase reproducibility. Secondly, seeds could be redried without promoting lethality. From the results obtained it is fairly safe to conclude that a postwashing period in the region of 12 hours can optimize the efficiency of EMS treatment. As observed from the study using a labelled mutagen postwashing of more than 12 hours would not be able to wash away more residual mutagen.

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Table 1: Influence of presoaking on effect of EMS treatment (0.2% EMS; 3 hrs treatment; 6 hrs postwashing)

Presoak (hrs)	Germination (%)	Seedling height (cm)	Seedling dry wt (g)	Survival (%)	Fertility (%)
control	98.0	26.5	1.37	86.3	95.5
0	93.0	24.1	1.09	86.3	83.3
6	89.7	25.8	1.21	89.0	90.6
12	76.3	25.9	1.18	71.0	93.3
24	86.7	25.6	1.31	84.0	89.5

Table 2: Calculation of efficiency of mutagen treatment based upon injury and sterility in M_1 generation and M_2 chlorophyll mutation frequency as influenced by different hrs of presoaking (0.2% EMS 6 hrs postwashing).

Presoak (hrs)	M_2 seedlings	Chlorophyll mutants (%)	Seedling Injury*	Efficiency	Lethality	Efficiency	Sterility	Efficiency
0	1.387	0.50	9.06	0.055	13.7	0.037	16.70	0.029
6	2,042	0.15	2.64	0.057	11.0	0.014	9.40	0.016
24	1,725	0.42	3.40	0.124	16.0	0.026	10.50	0.040

* Percent reduction in M_1 seedling height relative to control

Table 3: Effects of postwashing on growth parameters in M_1 (0.2% EMS; 3 hrs treatment; 6 hrs presoaking)

Postwash (hrs)	Germination (%)	Seedling height (cm)	Seedling dry wt (g)	Survival (%)	Fertility (%)
CONTROL	99.0	26.5	1.37	75.3	96.7
0	75.3	21.8	1.08	58.3	79.0
6	82.3	25.8	1.21	76.0	87.0
12	80.7	26.1	1.37	73.7	90.3
24	90.7	25.7	1.26	64.7	94.0

Table 4: Calculation of "efficiency of mutagen treatment" based upon seedling injury and sterility in M_1 generation and M_2 chlorophyll mutation frequency as influenced by different hrs of postwashing (0.2% EMS; 6 hrs presoaking)

Postwash (hrs)	M_2 seedlings	Chlorophyll mutants (%)	Injury*	Efficiency	Lethality	Efficiency	Sterility	Efficiency
0	944	0.32	17.74	0.018	41.7	0.008	21.0	0.015
6	3200	0.15	2.65	0.057	24.0	0.006	13.0	0.016
12	1261	0.47	1.51	0.311	73.7	0.006	9.7	0.049
24	2351	0.21	3.02	0.070	64.7	0.003	6.0	0.035

* Percent reduction in M_1 seedling height relative to control

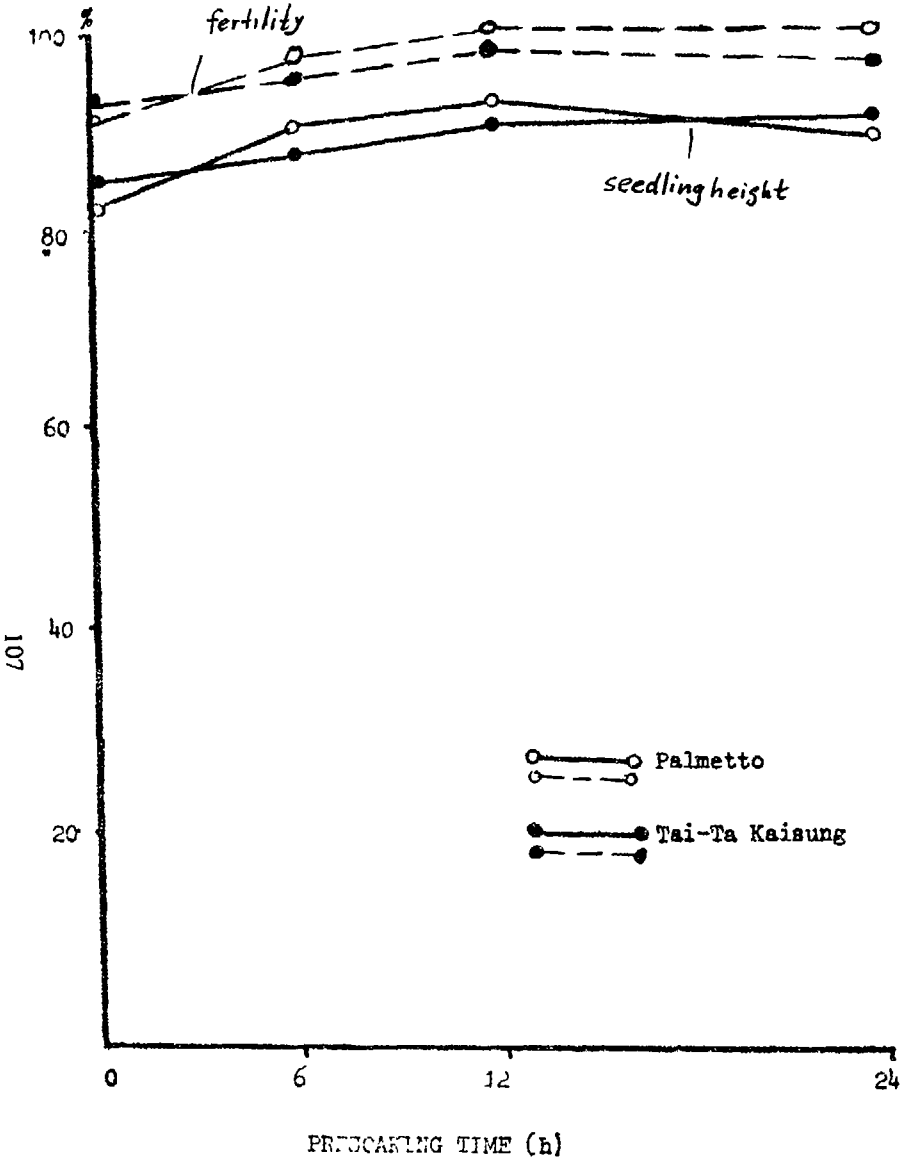

Fig 1 Effect of presoaking on the sensitivity of soybean embryos in M_1

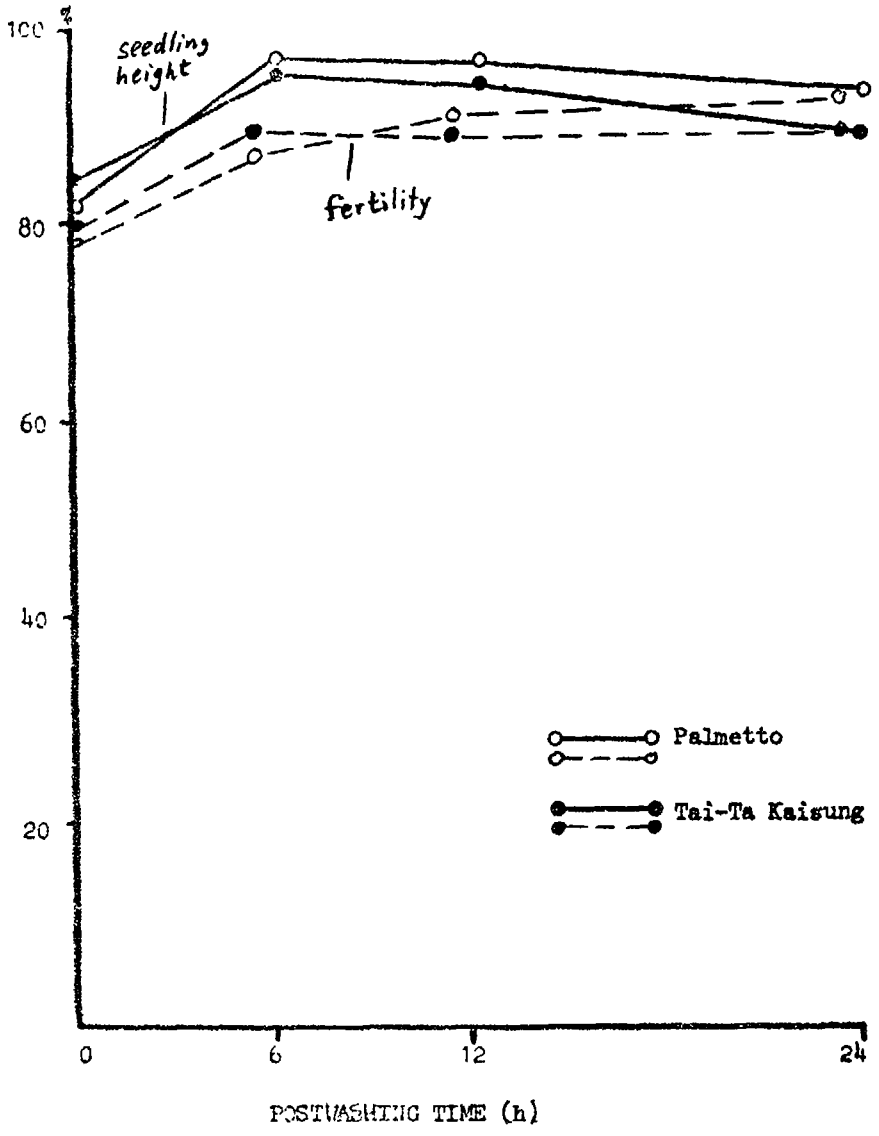

Fig.2 Effect of postwashing on the sensitivity of soybean embryos in M_1

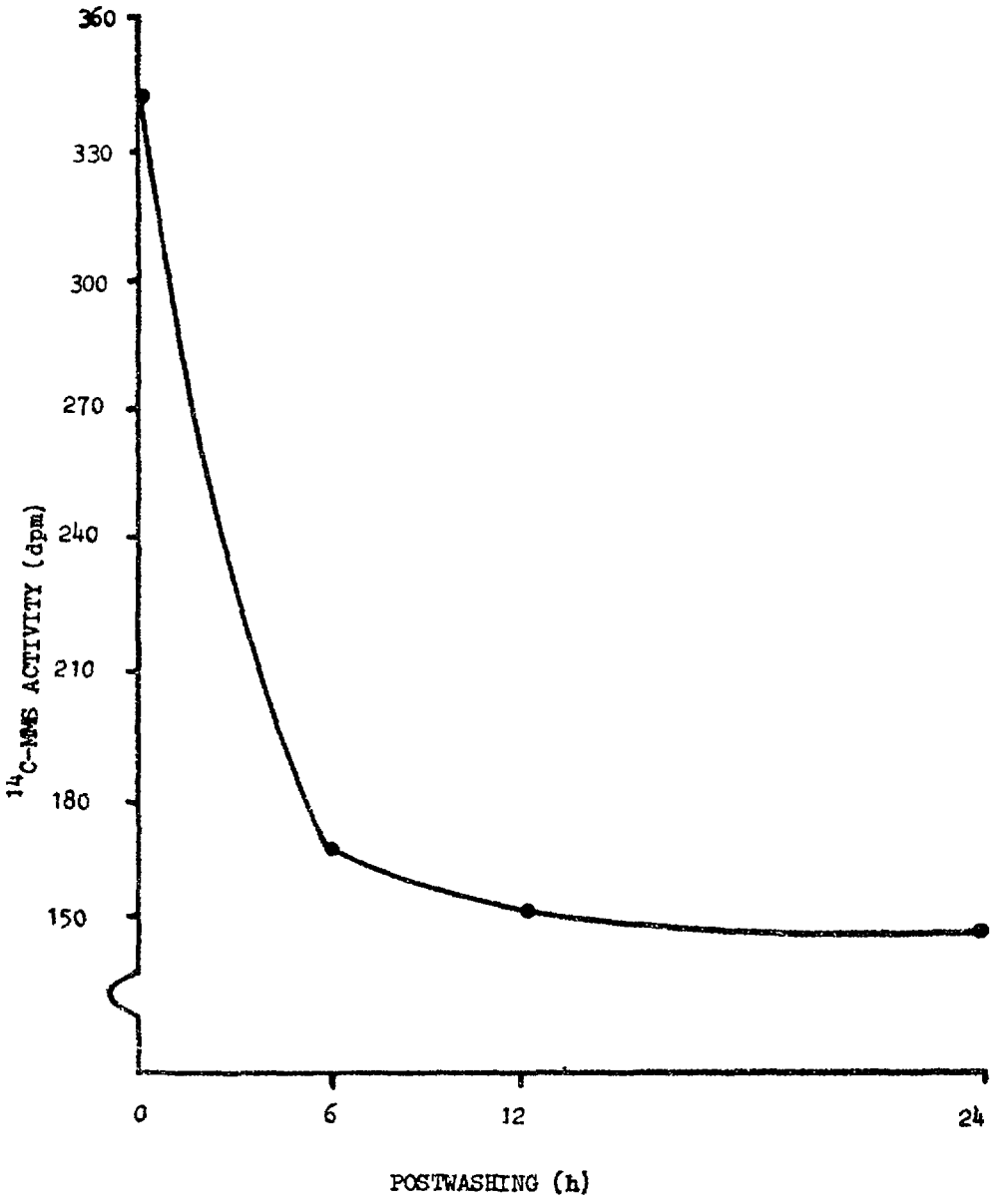

Fig. 3 Effect of postwashing on the elution of the mutagen from soybean embryos

MUTAGENESIS APPLIED TO THE IMPROVEMENT OF PHASEOLUS VULGARIS AS A GRAIN LEGUME CROP IN MALAYSIA

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Abstract

Phaseolus vulgaris is a common vegetable species in Malaysia, however its cultivation is costly and laborious, since well adapted types are climbing and require staking. A mutation induction experiment was started in order to check whether a non-winding mutant could be induced that is equally well suitable for humid tropic conditions.

Introduction

In Malaysia Phaseolus vulgaris is a crop of long standing. However, it is cultivated only for its immature pods which are marketted as French beans (pod vegetable). This very limited use and cultivation of the species locally is a poor reflection of the tremendous diversity and potential of the crop species which ranks among the world's oldest domesticated plants. This state of affairs in Asia is due mainly to the existence of other leguminous species which perform the same role as Ph. vulgaris does in its native Americas. However, in the present context of a fast increasing world population and diminishing resources particularly in the inadequate production of animal protein, the potential of such a species under local conditions should be reviewed.

Ph. vulgaris is generally believed to be "... not suited to the ever-wet tropics such as Malaya (Purseglove, 1968)". Considering the vast genetic resources of this species, it is our belief that there exist(s) population(s) which may be genetically disposed to Malaysian climatic conditions. This belief was put to test with the establishment of several field trials of acquisitions of Ph. vulgaris of the navy bean type. These field trials (1977-1979), tested 27 navy bean varieties from 10 countries in the Tropics (seed source - U.S.A.) and 7 varieties obtained from England (seed source - England). The varieties showed a range of growth habit types - determinate, bush and indeterminate climbing types. The conclusion from these trials is that only the

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climbing type showed good adaptibility to the local conditions in terms of general growth and yield. From among the climbing varieties tested there was one that showed remarkably good agronomic features. This was 209 - 480 from Nicaragua. The determinate and bush types showed poor adaptability. They exhibited poor growth and in the case of the variety Gratiot, flower abscission occurred resulting in zero yield even though vegetative growth was good.

Nicaragua 209 - 480 showed good agronomic features such as good establishment, short maturation period (33 days from sowing to flowering and 90 days from sowing to final harvest), good tolerance to diseases and pests, high podding ability, low podwall percentage, low shattering potential and fairly uniform seeding potential per pod. These and other characteristics are listed in Table 1. It is distinquished from the other varieties in having smaller flowers and smaller leaflets. The only agronomic feature that is considered undesirable is its growth habit. Cultivation of Nicaragua 209-480 in its present form would entail high labour cost in management (it requires staking) and at harvest (in one growing season it can support 3 harvest). Also, there is no agricultural system existing in the country that can include the cultivation of such a crop (climbing, grain legume crop).

In view of the good agronomic characteristics of this variety and the obstacles that would be encountered in trying to introduce this variety to the agricultural industry (mainly small holders and market farmers), the project to look into the use of irradiation as a means of changing the architecture of the plant was initiated. This project was embarked with great optimism because a mutation in the habit of Ph. vulgaris navy bean type was achieved and reported by Down and Anderson (1956)

As this project was started only in October 1980, the report would cover only the results of pilot test but the tentative schedule for the whole programme would be presented.

Materials and methods

The material for irradiation and planting is freshly produced seeds of Ph. vulgaris variety 209-480 from Nicaragua (referred to as Nicaragua 209-480).

The source of irradiation is a ^{60}Co gammatron supplying gamma rays at a rate of 393.5 rads/sec. located at Universiti Kebangsaan Malaysia.

Pilot Test I - To estimate the most suitable dose using seedling characters and percentage germination.

Seedlots consisting of 200 seeds each were irradiated with gamma rays according to the following doses - 0Krads, 10Krads, 20Krads, 30 Krads, 40 Krads and 100 Krads. The seedlots were then planted separately into boxes measuring 60 cm x 60 cm x 12 cm containing a soil mixture of earth, aged chicken dung and sand in the ratio of 3:2:1. (This would allow for replication over time and analysis using the ANOVA should the need arise). The boxes were maintained under greenhouse conditions until the cotyledons of the control plants (0Krads) were inclined to drop off at the slightest touch. The percentage germination for each treatment level was scored. The hypocotyl length and epicotyl length (per plant basis), fresh and dry weight (based on 3 random samples each of 30 randomly selected seedling per treatment), leaf area primary leaf (based on 3 random samples per treatment with each samples made up of 30 primary leaves i.e. from the sample of 30 random seedlings used for fresh and dry weight determinations) were measured. The data were analysed and are presented in Table 2 and Figures 1a and 1b.

Pilot Test II - To estimate the most suitable dose for large-scale irradiation based on field performance.

Seedlots consisting of 200 seeds each were irradiated with gamma rays according to the following doses - 0 Krads, 25 Krads, 30 Krads and 35 Krads. These were directly planted into the field at the rate of 200 seeds per bed. Each bed measured 10 m by 1 m and the seeds were planted in 2 rows with inter-row spacing of 0.6 m and inter-point spacing of approximately 10 cm. The beds were dressed with ground magnesium limestone at the rate of 1360 kg/ha and basal dressing of urea, muriate of potash and triple superphosphate at the rate of N : P_2O_5 : K_2O of 20:90:90 kg/ha. At the time of sowing Furadan 3G granules at the rate of 7.5 kg/ha were applied. Top dressing of urea at 10 kg/ha was applied in bands one month after sowing. The experimental design used was a complete randomised block consisting of 3 replicates each with 4 treatments and with each treatment randomly assigned to 1 bed. This plot was located in the Kawasan Penyelidikan, Jabatan Agronomi dan Kulturakebunan, Ladang Universiti Pertanian Malaysia in Serdang.

At the time of first harvest, the fourth mature pod from each fertile plant would be harvested and a single seed would be taken to contribute to the single seed bulk for each treatment level. The rest of the seeds would be bulked to form the single pod bulk for each of the treatment levels while the rest of the yield from the first harvest would be bulked to form the random seed bulk for each treatment level. These

would be used for the scoring of possible mutations in the M_2 generation should the need for such a study arise. The plants would not be maintained beyond the first harvest (expected after 66 days from sowing).

Results and Discussion

Based on the reports of various workers in the field of mutation breeding of leguminous crops (I.A.E.A., 1980) and particularly the report of Jalani (1977), the first pilot test with the objective of finding a suitable dose for gamma irradiation was designed. The range covered was from 0 Krads to 40 Krads with intervals of 10 Krads and with the inclusion of 100 Krads as a reference point. The radiation level that would give LD_{50} was expected to be approximately 25 Krads.

With reference to the results (Table 2), only the treatment level of 100 Krads showed significant decrease in 1% germination. In fact, based on the criterion of radicle growth the % germination was higher than the score of 21% which was based on the criterion of the cotyledons well raised above ground.

The other four treatment levels gave similar % germination scores. In fact, there did not seem to be any damage to the growing tip that was apparant up till 10 days after sowing when the treated plants showed twining behaviour similar to the control plants. However, visual differences in the intensity of green colour in the leaves were observed. The treatments which were irradiated appeared different in being uniformly covered with fine specks and the intensity of this effect appeared to increase with increasing dosage.

When the various treatments were subjected to a quantitative evaluation of the radiation effects on hypocotyl length, epicotyl length, fresh weight of whole plant, dry weight of whole plant, leaf area of primary leaf (Table 2), in general it can be concluded that the effects increased with increasing dosage.

Based on % germination, it was not possible to deduce the most suitable dose to use. It was deemed necessary to base it on some other criterion. The criterion used by Guhardja (1980) was approximately 30% reduction in seedling height. The response shown by the epicotyl appeared to be more sensitive (Figure 1a) particularly in the range 20 - 30 Krads. Based on this the appropriate dose would be 30 Krads.

To confirm this and also to assess the performance in the field so that the field requirements for large-scale trial can be estimated, the second pilot test was designed. The results of this are pending. It is hoped that based on the performance of the irradiated plants at selected

levels the appropriate dose can be determined and field requirements can be estimated more accurately.

With respect to the long-term use of the mutant if the project succeeds, direct use of the mutant is anticipated if there are no side effects resulting in poorer performance and reduced yield. If the side effects are present, hybridisation to the original type followed by selection is proposed.

The immediate step would be the test of true-to-type transmission of the mutant character, followed by seed multiplication of the mutant type and a small scale field trial.

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The Vice-Chancellor, the Dean of the Faculty of Agriculture and the Head and Staff of the Department of Agronomy and Horticulture for their interest, support and cooperation;

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To the following I would like to exoress my sincere thanks for their actual involvement : Mr. Baharuddin Jumani, Mr. Jamaluddin Bajuri, Mr. Abu Bakar, Mr. Abdul Hamid Shafii and Mr. Zahardin Zulkifli.

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Table 1 : Characteristics of Variety Nicaragua 209-480

Varietal Name	: 209 - 480	Country of Origin	: Nicaragua
Type of Bean	: Navy Bean	Colour of Bean	: White
Protein Content:	24%+	Moisture Content	: 12.5%
Growth Habit	: Indeterminate Climber		
Mutaration period (Days from sowing to first flower)	35		
Days to 1st Harvest	: 66#	% Yield Harvested	: 12.3#
Days to 2nd Harvest	: 84#	% Yield Harvested	: 66.8#
Days to 3rd Harvest	: 99#	% Yield Harvested	: 20.9#
Pod Yield per Plant	: 33 g#		
No Pods per Plant	: 34#		
Seed yield per Plant	: 26.7 g#		
No. seeds per Pod	: 5.1•		
% Shell	: 16.7•		
1,000 Seed Weight	: 138 g		
Nature of Pod wall	: Leathery, shatterproof		
Length of Pod	: 8.5 cm		
Colour of Flower	: White		
Shape of Pod	: Flat		
Total Pod Yield per Hectare	: 4,700 kg@		

+ Determined using Kjeldahl method and the factor 5.77.

• Determined using 12 samples ranging in size from 100 pods to 260 pods.

Determined using 3 samples ranging in size from 75 to 100 plants.

@ Estimates based on 3 beds (size 6 m x 1 m, seeding rate 10 cm spacing) and 8 beds (size 10 m x 1 m, seeding rate 20 cm spacing).

Table 2 : Results of Pilot Test 1

Gamma Irradiation (Krads)	% Germination	Seedling Length (cm) Mean	Hypocotyl Height (cm) Mean	s.d.	C.V.	Epicotyl Length (cm) Mean	s.d.	C.V.
0	98	25.0	10.7	0.78	7.3	14.3	1.17	8.2
10	99	25.3	11.5	0.85	7.4	13.8	1.19	8.6
20	100	23.9	10.4	0.74	7.1	13.5	1.30	9.6
30	99	19.9	9.4	0.93	9.9	10.5	1.96	18.7
40	97	12.3	7.3	1.36	18.6	5.0	2.36	47.2
100	21	-	-	-	-	-	-	-

	Primary Leaf area Mean (cm^2)	s.d.	C.V.	Fresh Weight (g) Mean	s.d.	C.V.	Dry Weight (g) Mean	s.d.	C.V.
0	16.0	0.67	4.2	4.17	0.58	1.4	4.26	0.18	4.3
10	14.4	0.74	5.1	40.7	0.58	1.4	3.86	0.04	1.1
20	14.5	0.53	3.6	40.0	1.00	2.5	3.91	0.08	2.1
30	13.2	0.16	1.2	37.3	1.15	3.1	3.85	0.07	1.8
40	10.6	0.15	1.5	31.0	1.00	3.2	3.35	1.06	3.2
100	-	-	-	-	-	-	-	-	-

Figure 1a. Effects of gamma radiation on seedling growth.

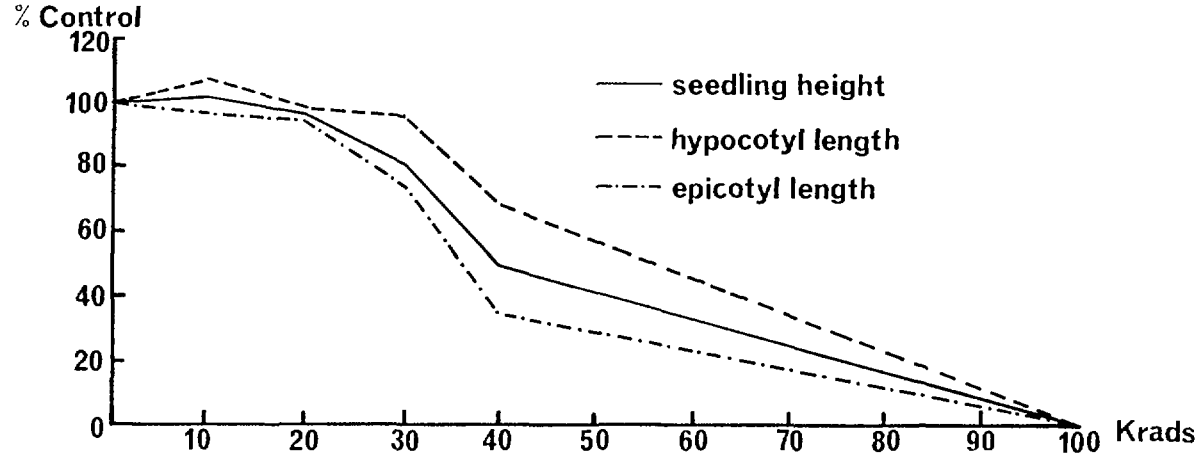
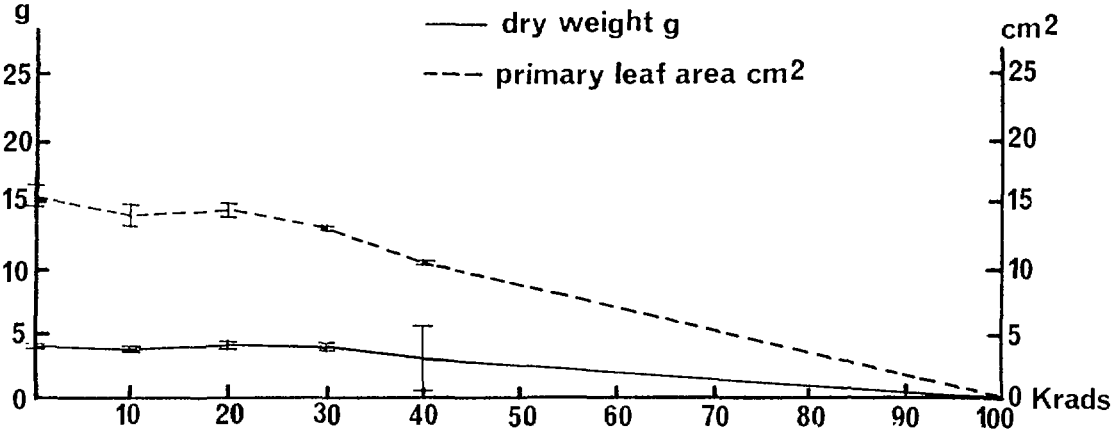


Figure 1b. Effects of gamma radiation on seedling growth



Programme

Scheduled		Executed
October 1980	Seed multiplication	23/10/80 - 10.2.81 (8 beds of 10 m x 1 m)
January 1981	Pilot Test 1	12/2/81 - 20/2/81
March 1981	Pilot Test 2	4/3/81
May 1981	Seed multiplication	
July 1981	M_1 Trial (5,000 - 10,000 seeds)	
September 1981	M_2 Trial (5,000 seeds single seed bulk)	

MUTATION BREEDING OF SOYBEAN FOR HIGH YIELD AND OIL CONTENT

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Abstract

Pakistan is facing a shortage of edible oils and a huge quantity is being imported every year. To improve local production, a mutation breeding project on soybeans was started in 1980 with the aim to induce and manipulate genetic variability for yield components, oil content, resistance to shattering, lodging and diseases in agronomically suitable varieties of soybean.

A preliminary report of mutagenic effects on the M_1 seedlings using gamma rays and ethyl methanesulphonate (EMS).

Introduction

Pakistan is deficient in edible oils to the tune of over 360,000 tons/year and to cope up with this demand a huge quantity is being imported every year. Serious efforts are needed to curtail the imports and save foreign exchange by enhancing the local production.

Among the oilseed crops recently introduced in Pakistan, particularly in Sind province, soybean has given encouraging results in commercial planting (Altaf, et al. 1977). It is a short duration crop, maturing in 90 - 120 days. The land is available in October for Rabi sowing. Thus agronomically soybean would fit in a soybean-wheat rotation to make better and intensive land use. Being a leguminous crop, soybean would also improve the soil fertility by supplying nitrogen from atmosphere.

The research work conducted on this crop has been mainly confined to evaluation of introduced varieties at the Agricultural Research Institute, Tarnab (N.W.F.P.) and the Agricultural Research Institute Tandojam (Sind). Breeding work in Pakistan on soybean is very scanty (Vasti and Keerio 1974). No systematic work for improving and yield components, resistance to shattering, lodging, diseases and for high oil content has yet been done.

At this centre, induced mutation breeding in soybean was initiated in 1980. The research project "Mutation Breeding of Soybean for High Yield and Oil Content" supported by IAEA under Research Contract No. 2673/RB intends to induce genetic variability for yield and yield components for resistance to shattering, and for oil content in soybean varieties suitable for Southern region of Pakistan.

Materials and methods

Homogeneous seeds of three varieties (Loppa, T-15 and Columbus) were treated with different doses of gamma radiation (0, 10, 15, 20 and 25 kR) and ethyl methanesulphonate (0.0, 0.25, 0.50 and 0.75%). The chemical mutagenesis was done for 3 hours after pre-soaking of seeds for 3 hours at room temperature, keeping in view other factors such as pH of solution, temperature, post treatment and washing, drying etc. The irradiated and chemically treated seeds were divided into two lots for laboratory and field studies.

Research supported by IAEA under Research Contract No. 2673/RB

Laboratory studies

From gamma rays and EMS treated material 100 seeds for each treatment were sown along with respective controls into sterilized soil in wooden boxes and placed in a glasshouse with the temperature ranging from 80 to 100°F. The seed emergence started on the 4th day of sowing in the gamma-irradiated material. Germination was delayed in all the treated populations as compared to the respective controls (Table 1.). However, there was no depressive effect of radiation on the germination percentage.

After two weeks seedlings were uprooted and rinsed with water for measurement. Measurements were recorded on the 10 longest seedlings from all the treatments for epicotyl length, hypocotyl length, seedling height (sum of epi- and hypocotyl length), fresh and dry weight. The different seedling growth parameters (Fig. 1 - 5) all indicate that with the increase of dose, the seedling growth decreased, but epicotyl length measurements seem to be more appropriate. Growth inhibition was stronger in the variety T-15 than for Loppa and Columbus. Reduced seedling growth with the increase of radiation dose is a common observation in barley (Conger and Stevenson 1969). Iqbal (1969) working with Capsicum annum observed that one of the effects of irradiation was the reduced mitotic frequency in the apical meristematic tissue. The retarded growth in our investigation could also be due to reduced mitotic division in the apical meristematic cells.

Primary leaves were generally reduced in size in the irradiated material with some difference among varieties (Fig. 6-8). Yellowish leaf spots were very common on primary leaves and their frequency increased with the radiation dose. Earlier Kazi (1972), Baradjanegara (1980) and Muszynski, et al. (1980), have reported a positive correlation between radiation dosage and no. of primary leaf spots. Blixt and Gelin (1964) have already linked primary leaf spots with the mutation frequency in Pisum. Therefore, the occurrence of primary leaf spots in the present study could be taken as indicator for a high mutation frequency.

Field studies

For field studies treated seeds were planted in a well prepared experimental field in a split plot design with four replications. The plot size was 3 x 2.25 m i.e., 5 rows of 3 m length. Seed was drilled at a row distance of 45 cm. Germination was recorded 2 weeks after sowing (Table 1.).

Plant to plant distance was standardized at 7.5 cm by thinning the crop before the first irrigation. Ten plants at random have been tagged from each replication of all the treatments for recording plant height, number of branches per plant, number of pods per plant, pod length, seeds per pod, 100 grain weight and grain yield per plant.

Future breeding strategy

During this year (June - July) the M_2 plant population will be carefully screened for all phenotypic variants ("macro"-mutants) and these will be harvested separately. Remaining, normal looking plants will be harvested either randomly or on the basis of good plant type. After threshing, seeds from each plant will be examined and those visibly different from the parent variety will be grouped into the "macro"-mutants. Estimation of oil content will be made on the M_3 seeds of both "macro" and "micro" mutants. Plants promising improved yield and yield components or hihg oil content will be carried forward to the next generation. Selected plants will be grown as plant progenies in the M_3 generation for confirmation of characters and for further screening.

In the M_4 generation, mutants of agronomic value will be evaluated in micro yield trials with two to four replications at two or more locations depending upon the availability of seed.

In the M_5 generation the best M_4 families will be used for multi-location yield trial in comparatively bigger plots.

In the M_6 through M_8 generations large scale testing at farmers' field, multiplication and vigorous screening will continue. Selected families will be few.

In the M_9 good lines/strains will hopefully be released for commercial growing.

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Table 1. Seed germination at 24 hours interval as affected by seed irradiation with gamma rays

Variety	Treatments	Days After Sowing									Germination Percentage	
		4	5	6	7	8	9	10	11	12	Lab.	Field
Columbus	Control	4	24	15	16	15	1	-	-	-	75	60
	10 kR	-	10	20	22	18	6	7	-	-	83	62
	15 kR	-	11	15	24	14	6	4	3	-	77	50
	20 kR	-	7	13	20	6	14	12	4	-	75	45
	25 kR	-	5	17	14	16	9	8	5	-	74	46
Loppa	Control	5	35	20	13	3	2	-	-	-	78	70
	10 kR	-	24	15	14	9	6	4	2	-	74	66
	15 kR	-	20	18	15	13	12	7	4	-	89	67
	20 kR	-	14	17	15	12	5	4	5	-	72	59
	25 kR	-	9	13	16	20	8	5	6	-	77	52
T - 15	Control	5	26	29	10	8	5	-	-	-	83	77
	10 kR	-	13	14	32	11	1	3	2	-	86	73
	15 kR	-	10	13	15	23	9	6	3	-	79	69
	20 kR	-	7	16	11	25	14	8	5	-	86	74
	25 kR	-	4	15	17	14	12	10	8	-	80	68

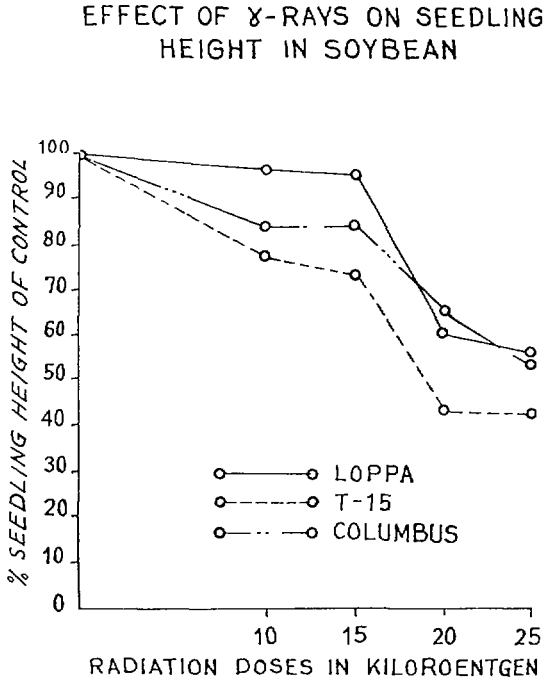

Fig. 1 EFFECT OF GAMMA RAYS ON SEEDLING HEIGHT IN SOYBEAN

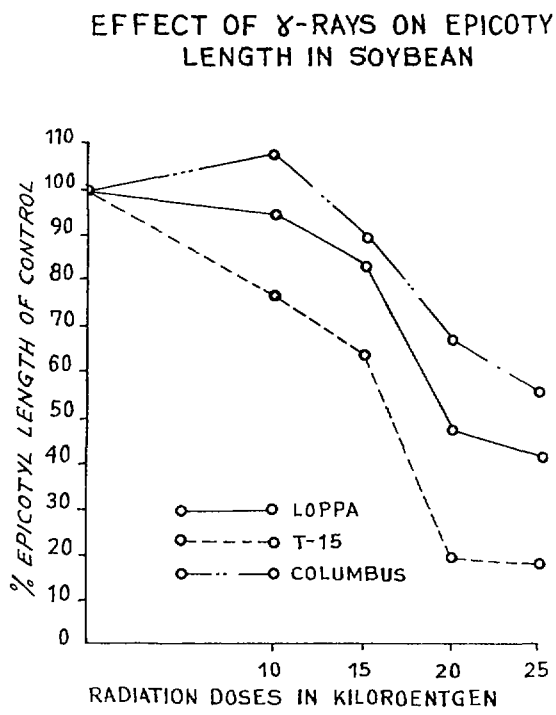

Fig. 2 EFFECT OF GAMMA RAYS ON EPICOTYL LENGTH IN SOYBEAN

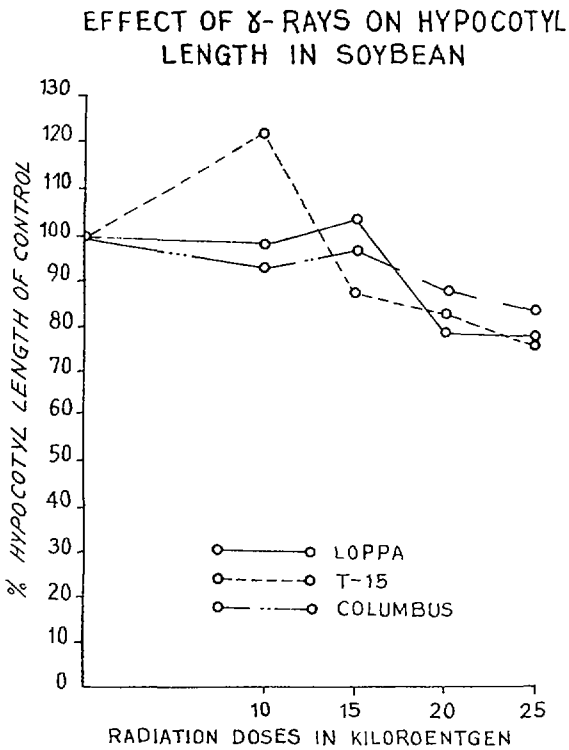

Fig. 3 EFFECT OF GAMMA RAYS ON HYPOCOTYL LENGTH IN SOYBEAN

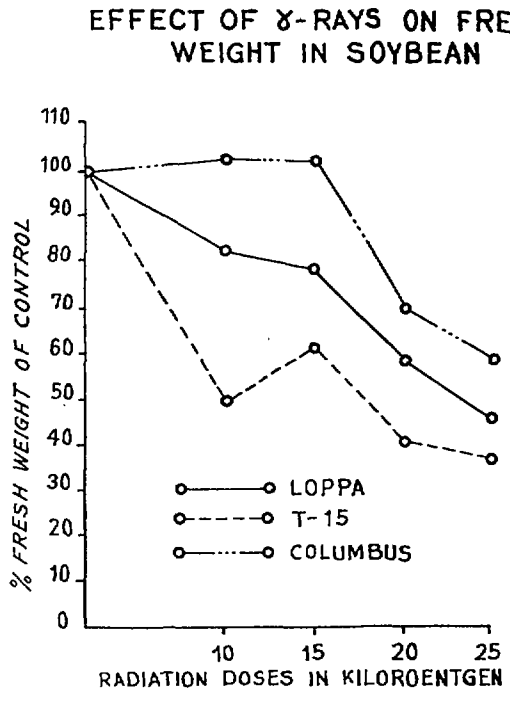

Fig. 4 EFFECT OF GAMMA RAYS ON FRESH WEIGHT IN SOYBEAN

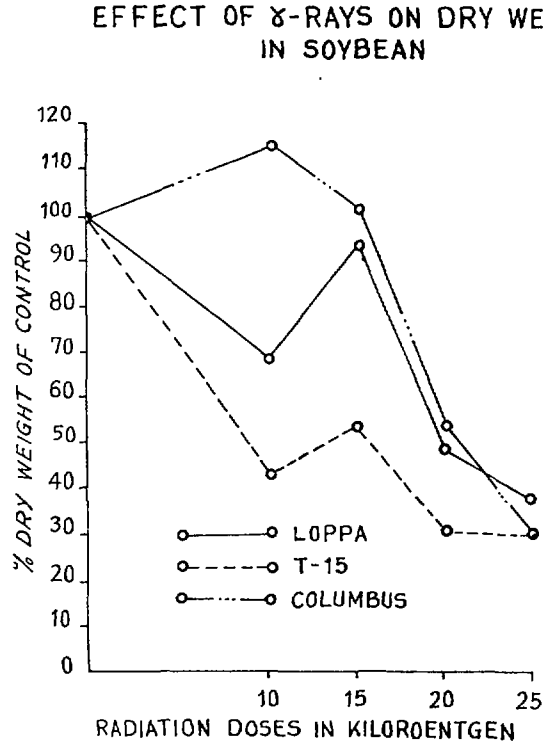

Fig. 5 EFFECT OF GAMMA RAYS ON DRY WEIGHT IN SOYBEAN

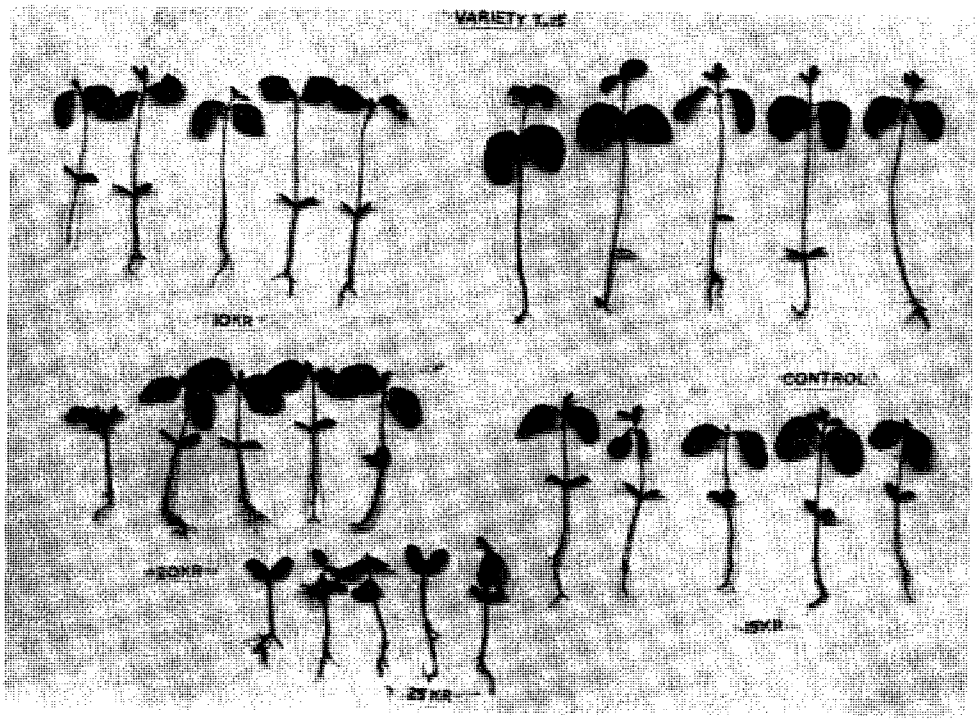

Fig. 6 M_1 SEEDLINGS OF THE VARIETY T 15

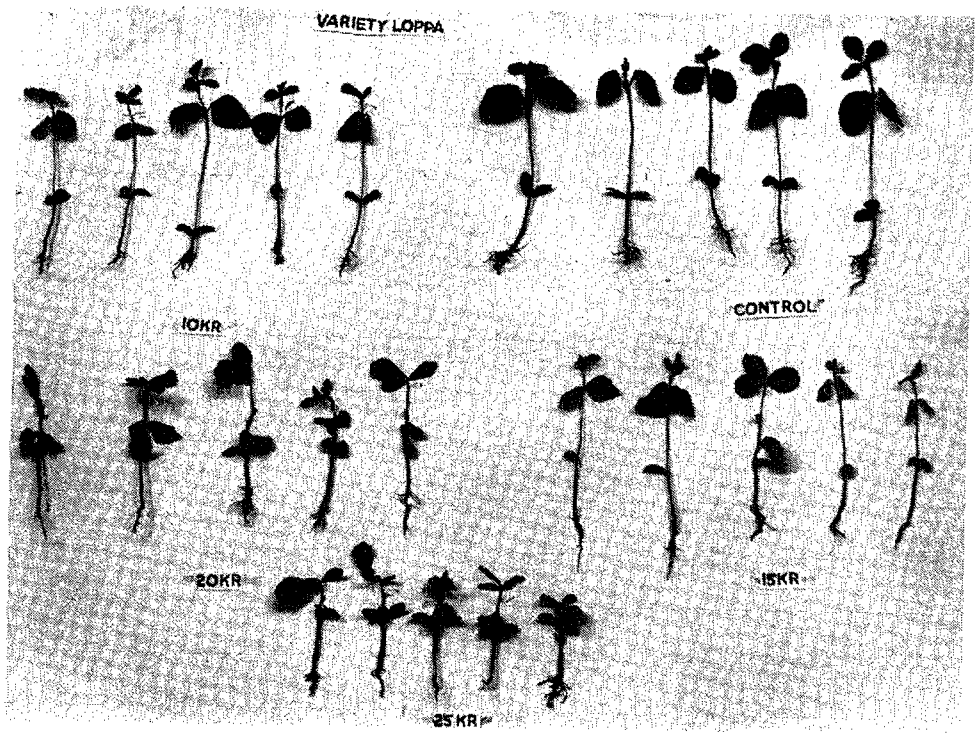

Fig. 7 M_1 SEEDLINGS OF THE VARIETY LOPPA

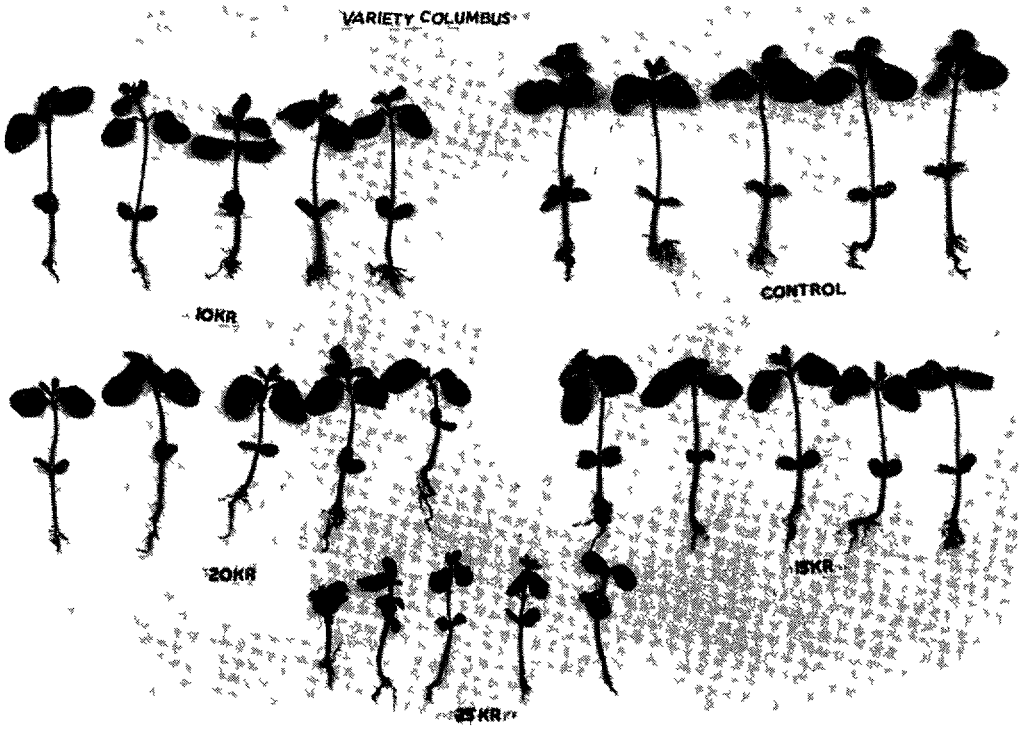

Fig. 8 M_1 SEEDLINGS OF THE VARIETY COLUMBUS

RESEARCH ON SOYBEAN MUTAGENESIS IN POLAND

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Abstract

The group of homozygous mutant lines of soybean was tested in 1980 with respect to important agronomic characters. High variability was found among the tested lines. Some of them would be included in future breeding programmesof soybean.

Introduction

Soybean is a very important source of plant protein and oil in the world. The cultivation area of soybean has been extended due to progress in modern plant breeding. This progress generated interest in soybean in countries of Central Europe, among them also in Poland. Early research on soybean in Poland was focussing mainly on the introduction and biological evaluation of foreign varieties. The next stage in soybean research began in the late sixties when recombination breeding of soybean started. At this stage collection and evaluation of soybean genotypes was intensified with the aim to select proper material for breeding.

First two Polish soybean varieties Ajma and IHAR 78/B were produced in the Institute. These varieties are characterized by good seed ripening and economically reasonable yield under the climatic conditions of Poland.

Mutation induction on soybean is undertaken in order to increase the genetic variability within this species and select proper parental material for breeding soybean varieties better adapted to climatic conditions of Poland.

Breeding methods and results

Mutation breeding of soybean started in 1974. Air dry seeds of varieties Warszawska, Ajma, strain "R", Acme and Chippewa 64 were irradiated with ^{60}Co gamma rays at doses of 4, 10, 13, 16 and 19 kR. The above soybean varieties were subjected in the next year to chemical mutagen treatment with NMH at concentrations of 1 and 2 mM. Seeds were pre-soaked for 16 hours in water and then treated with the mutagen for 3 hours.

The results obtained suggest that the applied doses should be changed: higher doses of both ^{60}Co gamma rays and NMH caused a significant delay in developmental phases in M_1 plants and high lethality. It was speculated that because mutagens caused delay in plant maturation and decrease in its productivity, lower doses of mutagen than suggested in literature might be used in our breeding programme.

The variability of quantitative characters in the M_2 generation was studied as well as the relationship between survival and productivity of M_1 plants vs. variability among M_2 and M_3 plants. This research was performed with respect to each mutagen used and dose applied.

Research supported by IAEA under Research Contract No. 2660/RB

M_1 plants with sufficient productivity were harvested and then multiplied in single rows, others were pooled together and multiplied in bulk up to M_3 using a modified single seed procedure. Selection started in M_2 using the length of the vegetation period, not longer than in parental lines, as a main character in both family-row and bulk populations. An assessment of mutation frequency and spectra was not undertaken. The theoretical and achieved breeding progress after selection was estimated in the period 1976-1978 in mutants with shorter vegetation period than the original varieties Warszawska, Ajma and strain "R". Generally accepted mathematical models were used. Besides the above group of mutants other mutants with interesting characters were isolated. Among them were dense pubescent mutants. This character appeared to be controlled by one dominant gene. Other mutants were: chlorophyllic, narrow-leaved and very early mutants selected from varieties Warszawska, Ajma and strain "R" treated with NMH. Several mutants with yellow or brown seed coat as well as a determinate growth type mutant were selected from the black seeded variety Ajma. Further research on them is underway.

Homogenous mutant lines, selected in M_2 and later from Warszawska, Ajma and strain "R" were tested in trials in 1980. Some lines are interesting as breeding material. Data characterizing 52 mutant lines compared with mother varieties Warszawka and Ajma are presented in Tab. 1. The new variety IHAR 78/B was additionally included in this test in 1980. Some of the tested mutant lines are better than varieties Warszawska, Ajma and IHAR 78/B as a check with respect to: plant height, location of the lowest pod, number and weight of seeds per plant. The earliest mutant line has a vegetation period similar to IHAR 78/B, many other mutant lines were earlier than varieties Ajma and Warszawska.

Data in Tab. 1. do not show clearly the variability among the mutant lines, therefore for each character studied classes of values were established and the number of lines in each class is present in fig. 1 A,B,C,D,E.

In over 17% of mutant linesplants were equal or taller than 66 cm, while the average plant height in 1980 for the varieties Warszawska, Ajma and IHAR 78/B was 40, 61 and 34 cm respectively. The lowest pod was located higher than 10 cm above the ground in 27% of the mutants. The number of seeds per plant was 31-40 for 27% of the mutant lines and in 10% of the lines it was higher than 41, which is more than the average for the mother varieties. With respect to seed weight per plant, 37% of the mutant lines were better than the parental varieties. The length of the vegetation period, the most important character for Poland, was between 130-150 days in 40% of the mutant lines as compared with 162, 160 and 130 days for Warszawska, Ajma and IHAR 78/B, respectively.

However, 37% of the mutant lines have a longer vegetation period, over 160 days. Among them are dense pubescent, chlorophyllic, narrow-leaved and mutants with only light coloured seed coat. These mutants can be used in breeding after detailed genetical studies.

Results presented above show that the mutant lines of sovbean obtained during the period 1974-1980 are very different. This variability of characters and its recombination in soybean cross breeding can certainly contribute to the progress in development of better varieties of this crop.

Future work will include evaluation of yield. Parallel to this, new irradiated materials will be planted in the coming season. Since November 1, 1980 part of this research is performed under contract with IAEA no. 2660/RB.

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Table 1 Main agronomic characters of soybean mutant lines as compared with parental varieties

Variety	Plant height		Location of lowest pod		Number of seeds per plant		Seed weight per plant		Vegetation period	
	$\bar{x}$	range for lines	$\bar{x}$	range for lines	$\bar{x}$	range for lines	$\bar{x}$	range for lines	$\bar{x}$	range for lines
Warszawska	40,1		7,7		20,7		4,2		162	
Ajma	60,7		7,1		28,2		4,8		160	
IHAR 78/B	33,8		7,5		37,8		7,5		130	
Mutant lines /52/	53,9	32,0	9,0	4,5	31,2	13,3	5,0	2,6	161	128
		73,0		15,0		50,4		9,4		178

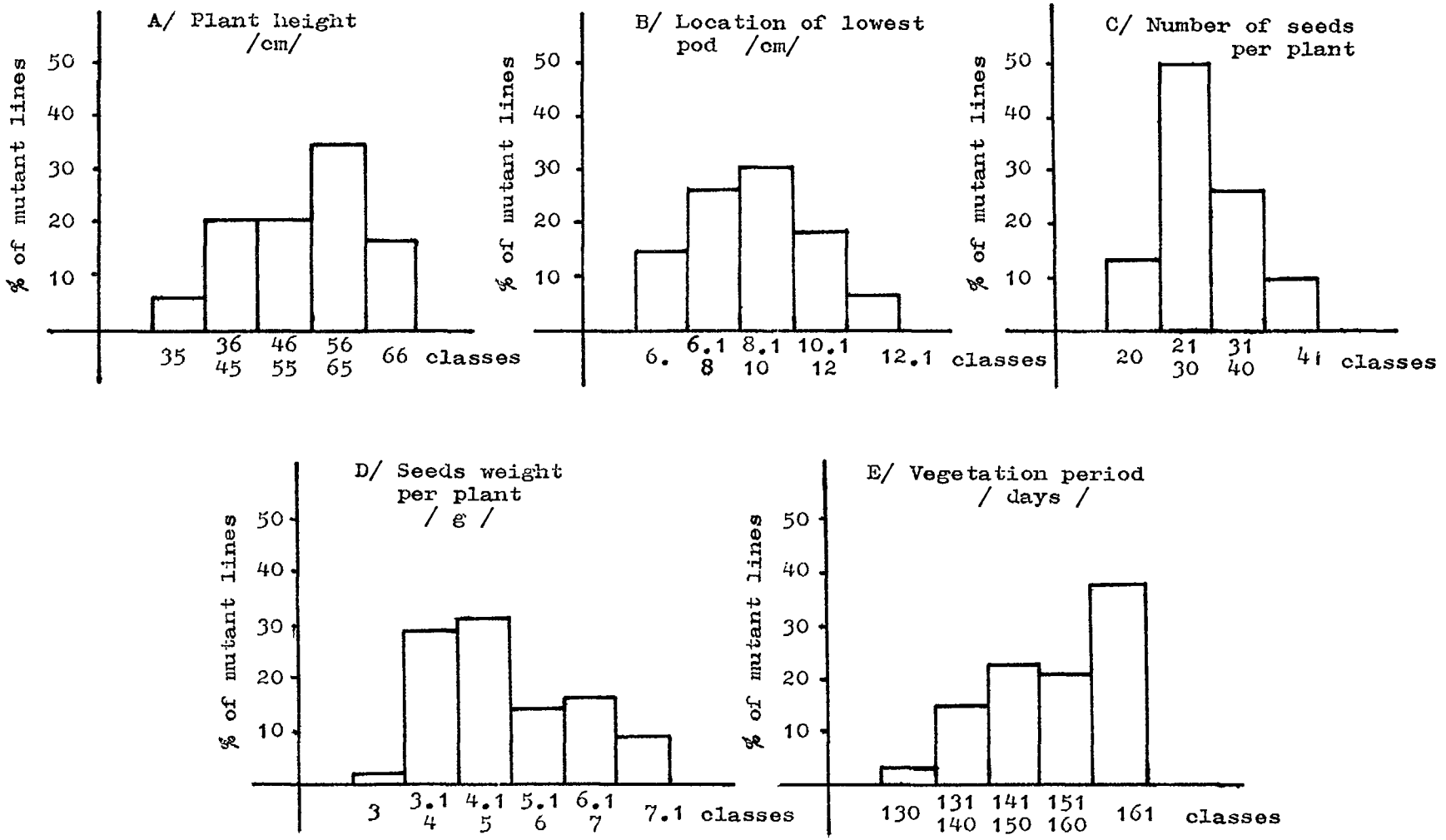

Fig. 1. Frequency of Mutant lines [percent] in different classes for each agronomic character tested in 1980.

EFFECT OF GAMMA RADIATION ON THE VARIABILITY OF SEED YIELD COMPONENTS OF GROUNDNUT (Arachis hypogaea L.) IN M_2*

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Abstract

A mutation induction experiment using ^{60}Co gamma irradiation was initiated in 1980 in order to improve adaptation of groundnut varieties to seasons of cultivation and cropping pattern. Considerable genetic variation was found in the M_2 generation and selection for improvement is being practised.

A major constraint in increasing the production of groundnut in the dry zone of Sri Lanka has been the lack of high yielding cultivars well adapted to different agroecological regions. Recommended varieties do not fit well into different seasons of cultivation and cropping patterns. The genotypic variability of agronomic characters in the germplasm collection was found to be low to moderate in a study undertaken in 1979 (Table 1). Therefore, with a view of inducing additional variability for quantitative characters, a mutation breeding programme was initiated in 1980. The objectives at the initial phase were to study the effect of varying doses of ^{60}Co gamma radiation on two cultivars and to evaluate selection response for seed yield components in the M_2 and following generations.

Materials and Methods

Air dried seeds of the cultivars GN 13 and Vietnam were subjected to 5, 10, 15, 20, 30 and 45 Krad of ^{60}Co gamma radiation at the Central Agricultural Research Institute, Peradeniya, Sri Lanka. 250 g of seeds were irradiated per variety per dose. Seeds were sown 40 hrs after irradiation along with the non-treated seeds in randomized blocks with three replicates. Percent emergence and survival were estimated in M_1.

Four seeds from every surviving M_1 plant collected from separate pods were bulked per treatment and grown in the "Maha" season, 1980/81. Sixty plants were randomly selected from each treatment for the estimation of the number of primary branches, dry plant weight, number of pods, pod weight, number of seeds, seed weight, yield (harvest) index, shelling percentage, number of seeds per pod and single seed weight. These procedures were followed for the un-irradiated controls of each variety. The remaining M_2 plants will be used for visual selection for seed yield components, particularly for characters with low modificational variability such as seed size and seed number per pod (Table 1). Variability of seed yield components in the M_2 of GN 13 cultivar is discussed in this paper.

* Supported by IAEA under Research Contract No. 2676/RB

Results and Discussion

The percent emergence and the percent survival of M_1 plants showed a tendency to decrease, with the increase of radiation dose (Table 2). However, percent survival decreased more rapidly with increasing dose of radiation. In both varieties, 50% survival under field conditions lies between 20 and 30 Krad treatments.

Table.1
Variability of Quantitative Characters in Groundnut

Characters	Range	CV_m, %	CV_g, %	H^2
Shelling percentage, %	60.3 - 74.6	8.9	6.0	0.31
Seed number	19.8 - 101	47.8	44.9	0.47
Pod weight, g	9.9 - 79.3	46.9	43.9	0.47
Pod number	13.5 - 66.1	49.1	45.3	0.46
Seeds per pod	1.47- 1.85	12.4	10.4	0.41
100 seed weight, g	34.6 - 58.9	12.1	18.8	0.71
Seed weight, g	15.8 - 62.7	47.7	44.1	0.46

The mean values of characters in the M_2 generation after seed irradiation showed a decrease in most of the treatments (Table 3). This can be attributed to the skewness to the left in the frequency distributions, commonly associated with the fitness characters in irradiated populations. However, the differences in the mean values in different treatments are fairly irregular. Thus, for shelling percentage, the mean values for low dosage levels were higher than the control. For pod number it showed a sharp decrease.

Table.2
Effect of gamma radiation dose on field emergence and survival of M_1 plants

Dose (Krad)	Emergence %		Survival, %	
	GN 13	Vietnam	GN 13	Vietnam
Control	76.5	74.3	89.8	90.7
5	72.7	70.1	78.9	79.2
10	70.6	69.9	76.5	72.3
15	67.1	70.8	71.0	64.9
20	69.2	64.3	60.9	63.1
30	65.0	65.1	46.7	41.3
45	64.6	62.3	32.1	36.7

Pod number and seed yield are highly correlated characters in groundnut (in our studies r_p= 0.912). The variation in the mean value in M_2 with increasing dose level of gamma irradiation, showed similar tendancies for these two characters.

Table. 3

Effect of gamma irradiation on the mean values ($\bar{X}$) of characters in M_2bulk populations

Treatment	Shelling percentage,%	Pod Number	Seeds per pod	100 seed weight,g	Seed weight, g
Control	68.1	34.7	1.70	51.6	19.0
5 Krad	72.5	27.7	1.70	50.9	18.2
10 Krad	70.4	23.9	1.64	49.5	18.0
15 Krad	72.0	18.2	1.69	50.4	14.9
20 Krad	66.1	35.9	1.68	34.2	20.7
30 Krad	64.8	29.9	1.68	34.7	16.2
45 Krad	68.2	33.7	1.57	45.5	24.5

The variation observed in the mean values of the identical character when subjected to different doses of gamma radiation prompted us to use coefficient of variation as the criterion for comparing the variability of characters in different M_2 populations. The data show that the variability of all the characters has more or less increased in the M_2 generation after seed irradiation (Table 4). Again, for the two correlated characters, i.e. pod number and seed weight, similar tendancies were observed in the pattern of change of variability. Highest variability for these characters was recorded in the 30 Krad treatment. Variability of the number of seeds per pod increased two folds in this treatment level. For 100 seed weight ,the variability reached its peak in the lower dosages of irradiation, i.e. 10 Krad and 20 Krad.

Table.4

Variability (CV,%) of characters in M_2 bulk populations

Treatment	Shelling percentage,%	Pod Number	Seeds per pod	100 seed weight	Seed weight
Control	10.2	41.4	7.6	15.8	35.7
5 Krad	12.1	31.4	11.1	18.3	34.5
10 Krad	11.4	42.8	11.1	20.9	33.9
15 Krad	11.2	49.1	10.2	12.1	43.1
20 Krad	13.2	49.0	10.6	20.9	46.8
30 Krad	12.8	58.7	16.1	17.8	57.6
45 Krad	12.3	50.1	14.9	15.4	54.4

Conclusions

When dry seeds of groundnut cultivars GN 13 and Vietnam were subjected to ^{60}Co gamma radiation, a decrease in the percent germination and percent survival was observed in M_1, with increasing radiation dose. 50% field survival, in both cultivars, was found to be in the range of 20-30 Krad.

When compared with the un-irradiated control, a decrease in the mean values and an increase in the variability of the seed yield components were observed in the M 2 bulk populations. These changes were more marked in the 15 - 30 Krad treatment levels.

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VARIETAL IMPROVEMENT OF MUNGBEAN AND BLACKGRAM THROUGH MUTATION BREEDING

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ABSTRACT

In order to enrich the available germplasm of mungbean and blackgram, a mutation induction experiment was started using ^{60}Co gamma rays. A number of promising mutants have been selected up to M_4 generation.

INTRODUCTION

Mungbean (Vigna radiata (L) Wilzcek) and blackgram (V. mungo (L) Hepper) rank high top among food legumes in Thailand in area, production and export value. In 1978 these two crops occupied an area of 400,000 hectares with a total production of 200,000 tons and export value of US$ 53 million. With growing world wide popularity of the two crops in the past decade, it is expected that demand would further increase. As a major exporting country, it is necessary that Thailand should be prepared to produce these legumes both in larger quantity and of higher quality.

A breeding programme on mungbean and blackgram started in 1979 and one variety of mungbean, U-Thong 1, was released in 1976. In the following year, U-Thong 2, a blackgram cultivar, was recommended. Their high yield potential, uniform pod set and maturity, non-shattering and large seed size made them highly acceptable to farmers and they are grown extensively at present. However, there are several characters that need improvement to make these two semi-domesticated legumes more reliable and profitable to farmers. Mutation breeding was initiated in 1979 to supplement the conventional breeding programme.

OBJECTIVES

Due to limited collection and non-availability of desirable characters in existing germplasm, mutation induction was taken up. The objectives of mutation breeding are as follows:

1. Plant Morphology

   1.1 Short stature and erect habit
   1.2 Determinate growth to encourage uniform maturity
   1.3 Non-shattering pod.

2. Physiological aspects

   2.1 Photoperiod insensitivity (day length neutrality)
   2.2 Early maturity
   2.3 Uniform flowering and pod setting
   2.4 Drought tolerance.

3. Resistance to major diseases

   3.1 Cercospora leaf spot
   3.2 Yellow mosaic virus.

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It is expected that some of the above specific characters would be available in the mutants which, if they also possess a wide and desirable agronomic base, may be directly utilizable, otherwise they will be incorporated into conventional breeding programmes. Even a small improvement of the above specific characters is believed to lead to increased production of the two legumes and considerable benefit to the farmers.

MATERIAL AND METHOD

Seeds of two standard varieties U-Thong 1 (mungbean) and U-Thong 2 (blackgram) chosen for this study were irradiated with gamma radiation at the Division of Radiation and Isotope, Kasetsart University, Bangkok, Thailand. Doses of 0, 30, 60 and 90 krad were initially tested to determine appropriate doses, using germination in the laboratory as a criterion. It was found that between 60 and 90 Krad were the best for U-Thong 1 and 90 to 120 Krad for U-Thong 2. For field testing one kilogram of seeds (about 15,000 to 16,000 seeds) were irradiated. Field trials were conducted at U-Thong Experiment Station 160 Kilometers west of Bangkok. Irradiated seeds were sown on September 5, 1979 in rows of 60 cm wide and 20 cm within row with three seeds per hill. Supplemental irrigation and plant protection measures were given whenever necessary to insure normal growth.

Data were collected on the plant stands, plant height, number of nodes and branches as well as other characters. U-Thong 1 matured in about 65 days and U-Thong 2 in 90 days.

To study the difference, if any, between pods borne on the lower and higher branches, pods from the first two lower branches were harvested separately. Due to smaller number of pods borne on the lower branches, three seeds per pod were randomly chosen from lower branches and one seed per pod from the upper branches. Seeds of M_1 plant from each treatment of both crops were carried forward to M_2 generation.

RESULTS

1. Mungbean

1.1. Laboratory observation

Table 1 shows the effect of radiation dose on germination, seedling height and root length 8 days after seeding. It should be noted that at a dose of 90 Krad roots failed to elongate.

1.2 Field experiment

M_1: Due to delayed onset of rain in 1979, irradiated seeds were sown on September 5, 1979 at U-Thong Experiment Station. Observations were made on five plant characters. Table 2 shows percentage of emergence, plant height, number of nodes on the main stem, number of branches and number of pods per plant. It should be noted that as the dose increased, emergence, plant height, number of nodes progressively decreased. On the other hand, the number of branches increased from 0 to 60 Krad and remained steady between 60 and 90 Krad. Interestingly, the maximum pod number per plant was attained at 60 Krad.

Treated plants were easily recognized by the numerous crinkled and chlorotic leaves. In addition, the M_1 plants tended to flower later and less synchronously, especially at the 90 Krad dose. Pod size increased and a higher proportion of empty seed could be seen in the treated plots. Incidentally, Cercospora leaf spot infection seemed to be low and later in M_1 population.

Table 1. Effect of radiation dose on mungbean*: germination, seedling height and root elongation (laboratory study)

Doses (Krad)	% of germination	Seedling ht. (cm)	Root elongation (cm)
0	99.67	7.46	4.56
30	91.33	6.18	4.41
60	79.67	2.84	1.78
90	38.33	1.52	-

* Measurements taken 8 days after sowing on 100 seedlings replicated 4 times.

Table 2. Effect of radiation dose on mungbean: emergence, plant height, number of nodes (on main stem), number of branches and number of pods per plant (Field experiment)

Dose (Krad)	Germination 1/ (%)	Plant ht. 2/ (cm)	no. of nodes 2/	no. of branches 2/ per plant	no. of pod 2/ per plant
0	61.81	52.64	11.26	1.37	14.11
60	25.02	48.87	11.43	2.43	21.39
90	0.80	33.00	9.85	2.41	11.24

1/ Counted 8 days after sowing in the field

2/ Average of 5 rows replicated 4 times. Each row consisted of 5 plants. Measurements taken at the time of harvest.

M_2 generation: M_2 seeds randomly selected from M_1 plants were planted at Chainat Rice Experiment Station on February 7 and harvested on April 21, 1980. Observations were made on germination, seedling height, plant height, nodes per plant, branches per plant, pods per plant, and seeds per plant. The results are summarized in Table 3.

Table 3. Mean values of morphological and performance data of M_2 plants following gamma irradiation compared with the control (U-Thong 1 mungbean). Chainat Rice Experiment Station, 1980 dry season

Doses Krads		Germ. at 2wk. (%)	Seedling ht. 10 days (cm)	Plant ht. at harvest (cm)	Nodes per plant	Branch 2/ per plant	Pods per plant	Seeds per plant
0		80.00	8.36	54.57	7.40	0.43	11.48	9.75
60	1* 1/	76.37	8.10	57.48	8.25	1.27	19.86	9.39
	2*	77.80	8.46	58.36	8.66	1.67	18.93	8.93
	top	75.28	8.31	60.41	7.70	1.03	16.57	9.41
90	1*	81.88	8.17	60.65	7.96	1.14	15.99	9.43
	2*	79.69	7.98	56.86	7.78	1.55	19.62	8.63
	top	79.75	7.85	56.91	8.83	1.73	21.78	8.81

1/ 1* and 2* M_2 plants raised from seed collected from 1st and 2nd lateral branches of M_1 plants.

2/ Lateral branch only.

Rates of yellow and trifoliate seedlings raised from seeds of M_1 plants collected from 1st branch, 2nd branch and top branches for yellow seedling and trifoliate 1st leaf are given in Table 4. The differences in mutation rate due to position of M_2 seeds on branches of M_1 plant are not significant. Other mutants found were tetrafoliate first leaf, dwarf plant, abnormal slender and tall plant, branching below first unifoliate and narrow leaf, which were mostly sterile.

Table 4. Mutation rate (%) of yellow seedling and trifoliate first observed in M_2 generation of 60 and 90 kilorad gamma irradiated U-Thong 1 mungbean plants derived from the seeds harvested from different part of M_1 plants

Seeds from	Mutation rate (%)			
	Yellow seedling		Trifoliate 1st leaf	
	60 Krad	90 Krad	60 Krad	90 Krad
1st branch	0.62	0.10	0.16	0.31
2nd branch	0.45	0.10	0.49	0.52
Top branches of M_1 plant	0.30	0.67	0.28	0.29
Average	0.46	0.29	0.31	0.37

M_3: Seeds of M_2 plants were randomly selected for study of 200-300 plants per treatment in the M_3 generation at U-Thong Field Crops Experiment Station during early rainy season (May - July 1980). Plants with desirable agronomic characters, as listed in the proposal, were selected in this generation. The results were as follows:

12 lines from 60 Krad 1st branch
8 lines from 60 Krad 2nd branch
39 lines from 60 Krad top branch
6 lines from 90 Krad 1st branch
5 lines from 90 Krad 2nd branch
15 lines from 90 Krad top branches

Data obtained for these selected lines are given in Table 5.

Table 5. Selected characters of M_3 plants to be carried forward to M_4 generation. (Mean in the upper lines are ranges in the brackets).

Sources		Days to flow.	Days to hvest.	Plt. Ht. (cm.)	No. Br. /plt.	No. pod /plt.	seed wt. /plt. (gm.)
60 kr.	1st b.	30.4	54.2	54.4	1.9	13.2	7.0
		(27-33)	(48-57)	(44-72)	(1.0-3.0)	(9.6-17.0)	(4.2-8.9)
	2nd b.	30.5	57.2	49.1	1.9	8.6	5.4
		(29-33)	(56-58)	(41-50)	(1.4-2.8)	(6.8-10.2)	(4.1-6.3)
	tb.	30.3	56.3	56.6	2.2	12.5	7.0
		(27-33)	(53-60)	(41-73)	(0.6-3.4)	(7.4-21.6)	(3.6-8.9)
90 kr.	1st b.	31.3	57.5	57.5	2.2	10.5	5.9
		(30-33)	(57-59)	(47-65)	(1.6-3.4)	(9.5-11.2)	(4.7-7.1)
	2nd b.	30.4	56.4	53.5	2.6	11.9	5.2
		(29-32)	(53-58)	(43-67)	(1.8-3.0)	(10.2-17.8)	(4.3-6.1)
	tb.	31.9	58.6	54.3	2.3	14.2	7.8
		(29-34)	(57-60)	(38-72)	(0.6-3.6)	(8.2-25.0)	(4.1-14.7)
control		32	62	86.8	1.45	13.2	9.38

It is evident that the mean plant height of selected M_3 plants tended to be shorter than control. This is a desirable trait to prevent lodging. Earliness in flowering and maturity are also an advantage in developing cropping systems. More number of branches and pods per plants are desirable yield components. However, lower seed weight per plant than the control suggests that the selected lines should be utilized only as breeding stock in a cross breeding programme.

Another set of lines was selected for high yield potential, even though they were somewhat late maturing. Six lines, like line 235, gave double the yield of control (Table 6).

Table 6. Data on earliness and seed weight per plants of some selected lines.

Lines no.	Source	Days to flow.	Days to hvest	Seed Wt. /pt (cm.)	Plt. Ht. (cm.)
235	60 Krad 2nd b.	29	60	18.8	56.6
441	60 Krad top b.	28	60	17.0	47.2
221	90 Krad top b.	30	58	16.7	67.8
25	90 Krad top b.	29	60	17.4	65.0
Control		32	62	9.4	86.8

M_4: Quantitative variations had been observed in M_3 lines in several characters such as days to flowering and maturity, plant height and others; it is necessary to apply further selection pressure. About 18 plants with promising characters from each treatment were chosen

and planted in M_4 generation during late rainy season (September - November 1980). The material was studied for earliness (Table 7) and yield potential (Table 8).

Table 7. Some characters of M_4 lines selected for earliness (September - November 1980 at U-Thong)

Line no.	Plt. Ht. (cm.)	Days to flow.	Days to hvest.	Seed Wt. per plt. (gm.)	1,000 SWT. (gm.)
Mean	78.5	28.7	53.4	11.7	53.2
Range	(52-89)	(24-31)	(50-57)	(8.9-21.0)	(34-71)
Control	83.7	33	64	7.7	62.2
19	81.6	31	55	9.9	71
55	86.6	26	51	21.1	52
96	84.6	29	51	16.7	62

Table 8. Yield components of M_4 promising lines selected for high yield (September - November 1980, at U-Thong)

Lines	Plt.HT. (cm.)	No. Branch /plt.	No. pod /plt.	Seed Wt. /plt. (gm.)	1,000 S Wt. (gm.)
Mean	78.5	2.3	20.1	11.7	53.2
Range	(52-104)	(0.1-3.2)	11.6-30.5	9.8-19.8	38-64
Control	83.7	0.94	11.7	7.7	62

Data in Table 7 and 8 indicate possibility of selecting for several desirable characters from these lines. M_5 generation was sown at Chainat in February 1981. Detailed observations are being taken to select uniform and stable material.

2. Blackgram

2.1 Laboratory

Germination count was taken 8 days after seeding and was found better than in mungbean even at a dose of 90 Krad (Table 9). As with mungbean, seedling height and root elongation decreased as doses increased. Even a dose of 90 Krad did not reduce the germination appreciably.

2.2 Field experiment

Only two doses, 90 Krad and 120 Krad were chosen for application since blackgram appeared to be less adversely affected by radiation. However, seeds treated with 120 Krad showed zero emergence. Data on plant survival and height only from control and 90 Krad are given in Table 10.

Plant growth and appearance were similar to those met with in mungbean.

Table 9. Effect of dose on blackgram: germination seedling height and root elongation (laboratory study).*

Doses (Krad)	Germination (%)	Seedling Height (cm)	Root elongation (cm)
0	93.33	6.79	3.03
30	97.00	6.24	2.76
60	92.00	4.74	2.52
90	89.33	2.17	1.25

* Average of 3 replications 100 seeds each, taken 8 days after sowing.

Table 10. Effect of doses on blackgram emergence, survival and plant height at harvest.

Dose (Krad)	Germination 1/ (% after 8 days)	Survival 2/ (% at harvest)	Plant Height (cm)
0	86.5	82.7	42.43
90	17.6	70.4	30.37
120	-	-	-

1/ Average from 25 sub plots, 4 square meters.

2/ Calculated from the difference between seedling count and stand at harvest. Average from 25 sub plots, 4 m^2 each.

M_1 : M_1 plants showed spot chlorosis in the first pair of true leaves and abnormal growth occured in the first and second trifoliate leaves (curl and ragged leaves). However, the spot chlorosis disappeared with subsequent growth and the subsequent trifoliate leaves were normal. The other abnormalities of

leaves were narrow and long leaf, and mosaic leaf. M_1 plants had a shorter internode compared to the control. Irradiation also induced variation in floral initiation: some M_1 plants were earlier and some were later than the control. In addition to the effect of irradiation on flowering time, pod shedding and sterility were also observed as in sorghum and cotton (Personal Communication, Jinda Jan-orn and Julee Tippayaruk, Field Crops Division, Department of Agriculture, 1980).

Table 11. Effect of irradiation of M_1 plants of black gram at the U-Thong Field Crops Experiment Station, 1979 rainy season.

Doses (K rad)	Germination % at 7 days		Field survival at harvest	Plant height at harvest
	Greenhouse	Field		
90	70.4	17.5	70.4	30.4
120	No record		0	0
0 (check)	96.0	86.5	82.7	42.8

The sets of M_2 seeds were planted at Chainat Field Crops Experiment Station in 1980 dry season and again at U-Thong Field Crops Experiment Station in 1980 early rainy season for studying mutation incidence in M_2 generation.

M_2 generation: M_2 plants showed various abnormalities such as, yellow seedling, trifoliate and tetrafoliate shapes of the first true leaf (unifoliate), narrow leaf alone and narrow leaf with twining of stem and unifoliate leaf and three-lobed unifoliate true leaf. The M_2 plants could be classified according to the leaf mutation as follows:

1) Narrow leaf: the leaf was narrow and long in shape

   1.1) Erect type: Short plants with dark green leaf, did not flower and set pods.

   1.2) Twining type: stem and branches were long and slender indicating climbing habit, and also possessed more branches. Some plants flowered and set pod but the seed aborted.

2) Unifoliate leaf: instead of forming normal trifoliate leaf after the first unifoliate leaf, plants formed unifoliate leaves at the nodes. Plants had several nodes with thick dark green leaf. Plants were thick and tall, green with purple tinge; there were white hairs on leaf and stem. Plants had several clusters of flowers at nodes, but they did not develop and were completely sterile.

3) Three-lobed unifoliate leaf: the leaf was light green in color with white hairs on the petiole, stem and pod. Plants were fertile, and able to set pods. Three-lobed unifoliate leaves occurred on upper part of stem while the leaflets of leaves in the middle part and lower part were still separate but borne from the same point.

Table 12. Mutation rate (%) of plant characteristics observed in M_2 generation from dose of 90 K rad of U-Thong 2 black gram, derived from the seeds harvested from different nodes (1^{st}, 2^{nd}, and top) of M_1 plant, Chainat Field Crops Experiment Station, 1980 dry season under irrigation.

Sources of Seed for M_2 Mutants	Yellow Seedling 1/ (%)	1^{st} leaf 2/		Narrow leaf 2/		Unifol. 2/ leaf (%)	3-lobed 2/ leaf (%)
		Tri-	Tetra	Twine	Erect		
1^{st} Node (1,600 seeds)	0.50	0	0	0	0	0	0
2^{nd} Node (2,400 seeds)	0.33	0.04	0	0.08	0.17	0	0.08
3^{rd} Node (3,200 seeds)	0.44	0.03	0.06	0.22	0.06	0	0
Top (4,000 seeds)	0.28	0	0	0.15	0.05	0.03	0.02
Control U-T 2	0	0.12	0	0	0	0	0

1/ Recorded at ten days after planting.

2/ Recorded at harvest.

From Table 12 it is not clear which part of M_1 plant yielded more M_2 mutants plants. It only seemed that the 2^{nd} node of M_1 plant yielded more erect plants with narrow leaf mutants compared to other parts. Seeds of M_1 plant from 1^{st} node (true leaf node) yielded no mutant in leaf characteristic. Therefore, it would be sufficient to collect M_1 seeds in bulk from the 2^{nd} node to the top individual plants.

Table 13. Certain morphological traits of M_2 mutants induced by the 90 K rad in comparison to the normal black gram, U-Thong 2, at U-Thong, 1980 late rainy season.[1/]

Traits	Normal	Mutants				
		Narrow leaf			Unifol. true leaf	lobed leaf
		Climbing		Erect		
		Sterile	Fertile			
Plant height (cm)	52.1	35.7	52.5	27.6	56.0	47.4
Branches per plant	3.9	5.5	7.0	7.6	4.0	7.0
Nodes per plant	15.3	14.5	12.2	10.0	19.0	15.5
Leaflet length (cm)	8.8	7.9	5.6	7.5	11.9	10.1
Leaflet width (cm)	5.4	2.5	2.0	2.2	9.7	9.2
Pods per plant	52.6	0	5.7	0	0	36.5
Pod length (cm)	5.3	0	3.6	0	0	4.6
Seeds per pod	6.4	0	5.0	0	0	5.6

1/ Data were taken at harvest. Six leaves and six pods were randomly selected for measurement.

Date showed that there are no significant diffence between control and randomly selected M_2 plants in these characters. Days to flowering and first ripening tend to be slightly shorter in the M_2 plants than control. M_2 plants which possessed desirable characters such as earliness, more branches and pods, seeds per pods and short stature were saved for studies in M_3 generation. A total of 800 lines (200 lines from each group) were carried forward from this season.

Table 14. Comparision between control and M_2 black gram on certain traits during February to May 1980 at Chainat.

Traits	Control	Seeds from 2/			
		1st node	2nd node	3rd node	top node
Germ. %[1/]	87.63	86.00	87.30	87.60	87.45
Seedling Ht. (cm.)	7.10	7.06	7.24	6.96	7.22
Plt. Ht. (cm.)	52.10	52.56	54.91	49.58	57.57
No. of branch	3.88	5.72	6.31	6.25	5.30
No. of pod/plt.	52.64	53.84	57.33	39.95	42.41
Days to flow.	37	33	32	31	32
Days to 1st pod ripen	57	51	51	51	51

1/ At 10 days after sowing, from 50 plants.

2/ Data taken from 8 rows and 5 plants per row selected randomly.

Unlike mungbean, blackgram is short day plant. In rainy season planting (long day period) flowering would initiate in October regardless of date of sowing. When planted early, that is, in May or June, the plants will grow vigorously and are seriously attacked by several diseases and insect pests. Thus, only few plants survive until flowering in October. In practice farmers prefer to plant in late rainy season, i.e., August to September in order to obtain better yield. Part of M_2 seeds were sown in early rainy season in attempt to find mutants that could set flower during this period. It was found that 261 plants bore flowers and set pods, indicating photoperiod insensitivity. Seeds from these plants were saved for verification in the next season.

M_3 : Another part of M_3 seeds (saved from selected M_2 plants) was sown in September 1980 at U-Thong. Evaluation for disirable plant characters was made among M_3 plants. A total of 57 plants were saved for study in M_4 generation. Data on certain plant traits are summarized in Table 15.

Table 15. Yield components and days to flower of selected M_3 plants obtained from September 1980 planting at U-Thong.

Sources (Seed from)		Plt. Ht. (cm.)	Branch /plt.	Pods /plt.	Days to flow.
1st node	Mean	68.5	9.19	127.9	33.5
	Range	(52.7-87.5)	(4.7-17.7)	(73.3-209.7)	(30-39)
2nd node	Mean	67.3	8.14	114.08	34.7
	Range	(46.0-88.5)	(4.3-13.7)	(77.0-189.3)	(32-37)
3rd node	Mean	63.9	7.98	116.44	34.7
	Range	(43.0-86.7)	(4.5-15.7)	(62.5-171.5)	(30-40)
Top	Mean	61.68	6.97	108.6	32.50
	Range	(35.2-81.7)	(3.2-12.7)	(55 -167)	(29.36)
Control		87.9	11.34	95.4	40

M_4 generation was sown at Chainat in February 1981. Evaluation and selection are underway for better performance, uniformity and stability.

Conclusion

Preliminary field data revealed the possibility of obtaining some better agronomic traits from M_4 plants of both mungbean and black gram. However, further studies are needed to confirm these findings. It may be too early to say whether these desirable mutants can be utilized directly in commercial production or incorporated into conventional breeding program. At least, the collection of material with promising agronomic characters has been enriched. In addition, other physiological traits such as drought tolerance, photoperiod insensitivity and resistances to major deseases will be tested in the future as special techniques needed for evaluation are not available at present.

INDUCED MUTATIONS FOR RUST RESISTANCE IN SOYBEAN

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Soybean rust, caused by Phakopsora pachyrhizi Syd. is a problem in many countries of South-East Asia. In Thailand, losses in yield through natural infection by this disease were 17.6 and 33.9 per cent in local cultivars, S.J. 1 and S.J. 2, respectively. Since 1971, a soybean improvement project has existed at Kasetsart University, Bangkok and efforts to develop productive soybean lines with rust resistance by induced mutations are also included.

Objective: This research project cosponsored by IAEA under Research Contract No. 2302/SD is aimed to develop higher productive soybean lines with high resistance to soybean rust by using gamma-rays as a tool.

Materials: Eleven cultivars namely: S.J. 2, S.J. 4, BM 50, BM 52, BM 90, G 8375, G 8377, G 8586, G 8587, Taichung N and Wakashima mutant no. 10 have been used in this experiment for mutation induction.

Location: The field experiments are being carried out at four locations as follows:

1. Kasetsart University, Bangkok.
2. Farm Suwan, Pakchong, Nakorn Rajchasima Province.
3. Mae Joe Experiment Station, Chiang Mai Province (18^{o} 30' N).
4. Nong Hoi Agriculture Experiment Station, Chiang Mai Province (ca. 1,000 m above the sea level).

Methods: Seeds with ca. 10 % moisture content were gamma irradiated at Kasetsart University with 15 and 30 krad. Irradiated seeds were planted in July, 1979 at Farm Suwan, ca. 10,000 treated seeds were used per cultivar per dose, besides 1,000 control seeds per cultivar.

At maturity time, M_1 plants were harvested as follows:

1. Ten plants from each treatment having good vigor were harvested and threshed separately to obtain single plant progenies for the M_2 generation.

2. Six pods from all other M_1 plants were randomly harvested and bulked, M_2-bulk seeds were obtained.

In dry season, 1980 (January - April), seeds for M_2-plant progenies and M_2-bulk were grown at Mae Joe Experiment Station for rust evaluation. Unfortunately, the rust infection in this season was almost nil, screening for rust resistance was impossible to be done. At maturity, M_2 plants were harvested as bulk, M_3-single and M_3-bulk were obtained.

M_3-single, M_3-bulk and the remnant seeds of M_2-bulk were grown at Mae Joe and Nong Hoi Experiment Stations, Chiang Mai in the rainy season of 1980 (July - October) for rust evaluation, using IWGSR rating system.

Research supported by IAEA under Research Contract No. 2302/SD.

Results: The natural rust infection at both locations in the rainy season of 1980 was very severe. By using the IWGSR rating system, 121 plants from different treatments which showed the low rating such as 323,333 were selected and threshed separately. Seeds of these selected plants were grown for seed multiplication at Kasetsart Experimental plot in the dry season of 1981 (January - April). All selected lines will be grown at Mae Joe and Nong Hoi Experiment Stations in the rainy season of 1981 (July - October) for further testing.

Note: For more information and details of the experiment, please refer to the first report on "Induced Mutations for Rust Resistance in Soybean" presented at the first coordination meeting on the use of induced mutations for improvement of grain legume production, held in Kuala Lumpur, Malaysia in 1979. A complete up to date report will be presented in June, 1981 in Denmark. At a FAO/IAEA Research Coordination Meeting on Induced Mutations for Disease Resistance in Crop Plants.

SPECIAL LECTURE

NITROGEN FIXATION POTENTIALS FOR IMPROVEMENT IN LEGUMES

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GENERAL

While there are many areas for improvement in legume productivity and agronomic performance eventually increasing the yields of well-grown, pest- and disease-free crops will require enhanced nitrogen input. N_2 fixation is an extremely complex process beginning with the successful colonization of effective Rhizobium strains in soil, their survival and infection of the host; followed by nodule development, bacteroid differentiation and the derepression of the unique enzymes of N_2 reduction, ammonia assimilation and the synthesis of translocatable solutes of nitrogen. The symbiosis is established when the host plant provides oxidizable carbon substrates to the nodule as a source of energy for fixation; the nodule in turn exports reduced nitrogen in the form of amino acids, amides or ureides for protein synthesis in the host. The functions of both partners are inter-dependent and considerable evidence has emphasised a direct link between rates of N_2 fixation and those of photosynthesis (Hardy & Havelka 1976). Thus nodule functioning might be limited by the supply of assimilates from the host and so improvement will necessitate either an increase in photosynthetic rate, a change in the partitioning of assimilates to the various competing 'sinks' on the plant or increased efficiency of assimilate use in nodule functioning. Recognising the potential for improvement in this area clearly demands an understanding of the physiology of N_2 fixation, photosynthesis and integrated legume growth.

It is important to recognize that increased N_2 fixation and thus N input to the crop can also be achieved by application of nitrogen fertilizer. Thus, from a physiological point of view, the rationale for increasing N_2 fixation requires that the energy demands for N_2 or NO_3^- utilisation are not greatly

different. From an economic point of view the high cost of fertilizer nitrogen may preclude its use in marginal and eventually in highly productive agricultural areas. It must be established however that plants receiving the greater proportion of their nitrogen from fixation do not suffer a yield penalty compared to those supplied adequate soil nitrogen. There is in fact a reciprocal relationship between atmospheric and soil nitrogen inputs in nodulated legumes and one potential for improvement lies in the selection of symbioses which maintain high rates of fixation in highly fertile, nitrogen-sufficient soils.

Nitrogen fixation occurs at widely differing rates during plant growth and is restricted to a period following nodule establishment and up to nodule senescence. Thus fixation can be increased by establishing functional nodules earlier following germination or by delaying senescence and extending their life through fruit development and seed filling. Nitrogen "hunger" during early seedling growth of legumes (especially of smaller seeded species) is well-established and while the cause and effects of nitrogen and carbohydrate limitation are not clearly established, Rhizobium strains differ both in their "infectiveness" and in the time required for nodule development. Similarly, the extent to which legumes show "self-destruction" (Sinclair & de Wit 1975) following anthesis varies markedly between species and cultivars of a single species (Phillips, 1980). While the nature of the signals controlling the complex of events which cause senescence in leaves and nodules is not well understood, seed development in legumes in general places a severe nitrogen demand on the plant with hydrolysis of functional leaf proteins resulting in declining photosynthesis and as a consequence, declining nitrogen fixation.

The final sentence of a recent review on the "Efficiency of Symbiotic Nitrogen Fixation in Legumes" by Phillips (Phillips, 1980) emphasises the complexity of the problem - "Enhancement of N_2 fixation in plant communities will require close cooperation between plant physiologists, microbial geneticists and plant breeders".

Biochemistry of Nitrogen Fixation in Legumes

Reduction of molecular nitrogen by the enzyme nitrogenase may be represented as:-

$$N_2 + 6e^- + 12ATP + 8H^+ \xrightarrow{Mg^{2+}} 2NH_4^+ + 12ADP + 12Pi \qquad -(1)$$

Both *in vitro* and in the intact nodule nitrogenase also reduces protons resulting in H_2 gas evolution concomittant with ammonia production.

Thus:-

$$2H^+ + 2e^- + 4ATP \xrightarrow{Mg^{2+}} H_2 + 4ADP + 4Pi \quad -(2)$$

The overall model equation for nitrogenase functioning in legume nodules may be represented as:-

$$N_2 + 16ATP + 8e^- + 10H^+ \xrightarrow{Mg^{2+}} 2NH_4^+ + H_2 + 16ADP + 16Pi \quad -(3)$$

Carbohydrate, mainly sucrose, formed in leaf photosynthesis is delivered in the downard-moving phloem stream to the root system and nodule of the legume. Oxidation of these substrates yields ATP and a source of electrons, probably as reduced ferredoxin or flavodoxin.

In a number of symbioses a bacteroid-located uptake hydrogenase utilises part or all of the H_2 produced (Schubert & Evans, 1976) and is generally considered to yield ATP from the oxidation (Dixon 1978). While this enzyme may function to prevent inhibition of nitrogenase by H_2 or to scavenge O_2 and protect nitrogenase from inactivation its activity recovers at least some of the energy lost to hydrogen production and increases the efficiency of energy use in the nodule. Theoretical considerations of the biochemistry of nitrogenase reactions indicate that if hydrogenase is absent (Hup^-) the expected cost will be 28-53 mol ATP/mol N_2 fixed; with complete recycling of evolved H_2 (Hup^+), this will be 25-39 mol ATP/mol N_2 fixed, or a saving of 11-26% of the energy required for nitrogenase functioning. While nitrogenase activity is but one of the energy-dependent processes of nodules, it is the major cost of respired carbon (see Table 1) and a conserving reaction such as that of uptake hydrogenase could, on theoretical grounds, cause a marked increase in the efficiency of photosynthate use in the nodules of those symbioses formed with naturally occuring or induced mutants of Rhizobium which are Hup^+. Field trials, in which soybean was nodulated with a number of Hup^+ and Hup^- Rhizobium japonicum strains, showed significant increases in plant dry weight and total plant nitrogen in those symbioses with the uptake hydrogenase (Albrecht et al 1979), so that this potential for increasing fixation may indeed be realised. The picture is complex however, and the size of yield increase is probably a function of which factors limit plant growth. In pot trials with cowpea nodulated with Hup^+ or Hup^- strains and in which light was non-limiting in an open canopy no difference in nitrogen fixed or yield was found between strains

(Rainbird, Atkins and Pate, unpublished). To date isogenic Rhizobium lines differing only in the Hup character have not been available so that the significance of uptake hydrogenase remains ambiguous.

Following excretion from the bacteroid to the host plant cell, NH_4^+ is assimilated initially to form glutamine and glutamate but eventually to form asparagine or the ureides, allantoin and allantoic acid. While this rather arbitrary division of legumes into "amide" or "ureide" producers may be too simple at present, "amide" producers are temperate species while those producing ureides are of tropical origin (Table 2). Considerations of the pathways of synthesis for each class of these exported solutes indicates that, while the pathways are very different, the cost in terms of energy is, in each case, about the same (Table 1). Thus, even though amides or ureides may be of some, to date unknown, advantage to the species in which they predominate, there is apparently little potential for improving nitrogen fixation by alteration of the end products formed in nodules. The anatomy of nodules formed in these two classes of legume is quite different. Those producing ureides have a closed vascular system which may be better adapted to exporting a high flux of solutes compared to the open non-continuous system in those producing amides (Sprent 1980). Increasing legume yields may require greater rates of transport of nitrogenous solutes and selection for nodule vasculature may be a useful trait in this respect.

Cost N_2 Fixation v Cost of NO_3^- Assimilation

Uncertainty about the partitioning of electrons between proton and nitrogen reduction and the extent to which uptake hydrogenase recycles electrons and recaptures ATP (see above) does not allow an estimate of the theoretical costs for nitrogenase *in vivo* except within defined limits. These are 11-35 mol ATP/mol NH_4^+ or 0.33-0.69 mol glucose/mol NH_4^+ (see Table 1). For the reduction of NO_3^- the reaction sequence involving nitrate and nitrite reductases is:-

$$NO_3^- + 2\bar{e} \longrightarrow NO_2^- \qquad (4)$$

$$NO_2^- + 3 \times 2\bar{e} \longrightarrow NH_4^+ \qquad (5)$$

That is 12 mol ATP/mol NH_4^+ or around 0.3 mol glucose. Thus, while the most efficient N_2 symbioses would be energetically similar to NO_3 reduction

many could be considerably more expensive. There is some evidence which indicates that NO_3^- uptake is an "active" process in plant roots depending on the functioning of a membrane bound corner mechanism linked to an ATP-ase (Butz & Jackson, 1977). In this case NO_3^- uptake would add to the costs of NO_3^- assimilation, but only to the extent of 1 mol ATP/mol NH_4^+ formed.

In some legume species (e.g. *Lupinus*), much of the incoming NO_3^- is reduced in roots and is assimilated into organic forms which are subsequently transported to the shoot in xylem (Atkins *et al.* 1979). In others (e.g. cowpea, soybean) most of the NO_3^- is transported directly to the sites of transpiration in the shoot and is reduced and assimilated in leaves (Atkins *et al.* 1980) by a light-linked sequence of reactions (Canvin & Atkins 1974). While reduction at heterotrophic sites in the plant would require the full cost (12-13 mol ATP/mol NH_4^+) at the expense of fixed carbon, in leaves, photosynthetically-generated reductant and ATP may be used directly thus significantly reducing the cost to the plant. In most legumes the extent to which NO_3^- is reduced by respiratory-linked reactions versus a sequence directly coupled to photosynthetic electron transport has not been defined. Furthermore, the notion that NO_3^- assimilation in tops is essentially "free" has not been clearly established and it is premature to suggest that plants reducing NO_3^- in this way will be more economic of their carbon resources than those in which NO_3^- is reduced in roots or those dependant on N_2 fixation.

Experimental approaches to the costs of N_2 and NO_3^- reduction have been complicated by non-comparability of plant material, differing rates of nitrogen accumulation and uncertainty about the siting of NO_3^- metabolism. A quantitative study of the C and N economy of nodulated and non-nodulated *Lupinus albus* over a short period in mid vegetative growth (10 days) is shown in Table 3. The carbon and nitrogen increments of each were similar during this period of growth and in the NO_3^--grown case 90% of the NO_3^- was reduced in the roots (See Atkins *et al.* 1979, Pate *et al.* 1979) allowing a valid comparison of the two types of plant. While the same ammount of carbon was translocated to the roots in each case, a greater proportion was lost as respiration in the nodulated plants (see Table 2). This difference was due however to increased respiration of the supporting root rather than to the nodules (Pate *et al.* 1979) indicating that the cost of assimilating

N_2 or NO_3^- was not significantly different. A similar study using nodulated and non-nodulated cowpea (Atkins *et al*. 1980 reached a similar conclusion.

Nitrogen Fixation and Photosynthesis

The role of photosynthesis in maintaining symbiotic nitrogen fixation has long been known; however, recent experiments have shown that increasing the rate of CO_2 fixation (by increasing CO_2 level or decreasing O_2 level) promotes a direct enhancement of nitrogen input (see Table 4) and may result in significant yield increases. However, selection of cultivars for increased photosynthetic rates, for reduced photorespiration or for "C_4" photosynthetic characteristics from naturally occurring populations or from cross-breeding programmes have not, to date, identified desirable variants with increased carbon fixation. Similarly, changes in ambient CO_2 or O_2 concentrations in the field are probably neither feasible nor practicable in the near future.

A second approach might be to increase the proportion of photosynthate which is allocated to nitrogen fixation either by changing the source/sink relations of the plant or by extending the persistence of fixation into seed filling. Quantitative C & N balance studies of a number of nodulated legumes have been used to assess the proportional distribution of photosynthate to nodules (see Table 5). The three species shown differ markedly in their allocation to nodules but also within each species a declining proportion is available to nitrogen fixation as growth proceeds to seed maturation. This is much more severe for the two tropical species than for *Lupinus albus*. In general however, nodule functions use around 10% of the plant's total carbon resources so that partitioning of a further 10% could be expected to double nitrogen input. This seems a small change but the consequences of such a manipulation, were it possible, cannot be assessed. While studies with cowpea in which the size and activity of 'sources' and 'sinks' on the plant have been modified, have revealed a high degree of plasticity in the pattern of allocation of assimilates from particular leaves to particular organs, alterations in sink strength are not readily made in the field and criteria for selection of plants with these traits are not obvious.

Considerable experimental evidence shows that artificial manipulation of the activity of sinks on a plant is followed by an adjustment of photo-

synthetic rate (Herold 1980). It is difficult to assess to what extent adjustment is a direct outcome of altered sink strength and to what extent it is simply an incidental effect due to the manipulation. Nevertheless, the "unimproved" plant apparently possesses considerable potential to increase source output if there is an increase in the demand for assimilates at the sinks. An example is shown in Tables 6 and 7 (unpublished data from Peoples, Atkins and Pate) where at anthesis in cowpea plants, leaf area was reduced by removing one or two leaflets at each trifoliolate or the distribution of photosynthetic surface was altered by whole leaf removal at one or a number of nodes. While diminution of leaf area reduced plant and seed dry matter yield compared to control plants (Table 6) the reductions were proportionately much lower than those of leaf removal For example, the one third reduction in area reduced total plant yield by 9% and seed yield by 7%; the two third reduction caused a 13% and 35% fall in plant and seed yield respectively. Similarly, significant leaf removal only caused a slight reduction in N_2 fixed at final harvest (Table 7). With one third leaf removed, only an 8% reduction occurred and with two third removed, fixed N fell by only 22%. Thus photosynthesis per unit leaf area (or mass) was increased significantly int the leaves remaining on the plant indicating that, in cowpea at least, productivity and N_2 fixation is not limited by photosynthetic potential *per se*.

The balance between source activity and sink demand is clearly linked by some translocated "message" and it seems reasonable to suppose that knowledge of the mechanism involved will indicate the likelihood of its being manipulated to increase "sink strength" and lead to enhanced photosynthesis.

The decline in nitrogen fixation following anthesis is accompanied by mobilisation from the vegetative structures of the plant to satisfy the nitrogen demands of seed development. In all plants the principal source of tissue nitrogen is the so-called "fraction-1 protein" of the leaf This protein, which comprises 50% or more of the soluble protein of the shoot, is however also responsible for the enzymic fixation of CO_2 in photosynthesis Thus its breakdown to yield nitrogen apparently leads to diminishing photosynthesis, rapid leaf senescence and "self destruction" (Sinclair and de Wit,

1975) of the plant's metabolic functions. Fraction 1 protein is preferentially degraded as leaf functioning declines (Fig. 1).

In general three sorts of explanation for post anthesis senescence have been proposed. In the first it is the competition between the phloem-feed sinks of the plant for assimilates which leads to nutrient diversion to the developing fruit (Nooden & Leopold, 1978). Their increasing "sink-strength" dominates the translocation patterns of the plant and results in nitrogen withdrawal from leaves. In the second a discrete "senescence signal", possibly hormonal, is translocated from the developing reproductive structures or is formed in other organs as a result of the presence of reproductive structures (Nooden and Leopold, 1978). Accumulation of this "signal" in vegetative tissues causes a decline in their metabolic functions and as a consequence senescence ensues and nitrogen is released for fruit development. The third explanation is that the plant has a defined growth potential and senesces at a particular age coincidental with the onset of fruiting. This theory is unlikely to apply to most legumes as fruit removal prolongs vegetative development (Nooden, 1980) and in some cases results in growth far beyond normal (Nooden 1980). To date, it has not been possible to clearly establish whether the first or second proposal is the more likely for legumes in general. Whether the events which trigger senescence are hormonal or are due to nutrient diversion could however have considerable bearing on the criteria used in selecting lines for plant breeding efforts to improve post anthesis performance.

Using the simple selection criterion of maintenance of "green" leaf colour at pod maturity Abu-Shakra *et al.* (1978) successfully selected soybean plants in the field for delayed leaf senescence. They demonstrated that these lines had a heritable component which maintained both carboxylation activity in the leaves and continuing N_2 fixation throughout seed filling. Incorporation of such traits into a desirable genetic background could help increase seed yield but to date such lines have not been produced.

Establishing the Symbiosis

For nitrogen fixation to occur in the field, there must be a sufficient number of *Rhizobia* in the soil such that infection of root hairs occur and effective nodules develop which persist during plant growth. There is a

fairly close interrelationship between the bacteria and host plant with considerable specificity existing both as to species and cultivar of legume and strain of *Rhizobium*. Due to a lack of highly effective bacteria in a soil or a low frequency of organisms due to poor survival in the soil, nodulation may be inadequate, or is achieved by relatively ineffective *Rhizobia*. In either case nitrogen fixation will be sub-optimal and inoculation of seed at planting with a highly effective culture of *Rhizobium* might be required.

Table 8 shows the main steps which are involved in the establishment of the symbiosis. While this list is probably simplistic, it does emphasise the need for a complex series of events which necessarily will require time for completion. Thus following germination of inoculated seed, 1 - 3 weeks are required before effective nitrogen-fixing nodules are formed. In this period the young seeding must rely on seed and soil nitrogen for protein synthesis and small-seeded legumes particularly often exhibit a pronounced "nitrogen hunger" at this time. Clearly, selection of symbioses in which functional nodules are established earlier could lead to increased seedling vigour and result in enhanced yield. Low levels of combined nitrogen, especially when applied as a "starter" fertilizer at germination, frequently stimulate plant growth and may even enhance nodule number and size, especially if nodule development has been delayed due to sub-optimal temperatures etc. (see Dart and Mercer, 1965).

Effects of Combined Nitrogen on Nodule Function

The interaction of combined nitrogen application and N_2 fixation is a complex one, which varies markedly with plant species, strain of infecting *Rhizobium*, plant age, form of combined nitrogen, site of nitrogen application to the plant and a series of environmental factors. Apart from the stimulatory effect of "starter" doses where nitrogen "hunger" occurs in young seedlings (see above), combined nitrogen is inhibitory to nitrogen fixation. Two basic classes of inhibitory effects have been described, the first relating to the initiation and development of nodules, the second to the nitrogen fixation activity of already functioning nodules. The mechanism of inhibition in each case has not been adequately defined and a range of explanations involving direct effects of NO_3^- or its reduction products

(NO_2^-, NH_4^+) or nodule initiation, development and nitrogenase activity to those of an indirect nature involving changes in hormone production and the competition for photosynthates within the plant have been postulated. In any case, there is generally a reciprocal relationship between the supply of combined nitrogen and N_2 fixation over the vegetative and reproductive periods of plant growth. Symbioses which are tolerant of high soil nitrogen levels and in which nodulation and subsequent nitrogen fixation occurs at high rates under these conditions are obviously desirable if N_2 fixation is to be maximised. Variation shown by both host and *Rhizobium* strain in altering the sensitivity of a symbioses to combined nitrogen indicates that there may indeed be considerable potential for improving the symbiotic performance of legumes under high fertility conditions.

Assessing Symbiotic Performance in the Field

Selection criteria for superior symbiotic performance in the field depends to some extent on whether or not the legume under test is to be nodulated by the *Rhizobium* strains native to the soil at that site or whether the seed is inoculated with a known *Rhizobium* culture which is highly effective on that legume. In either case two prerequisites should be observed:-

(1) The soil should be low in available N but adequate for other nutrients (even if added as fertilizer).

(2) Symbiotic performance should be gauged against a treatment in which plant growth and yield is maximised by the addition of fertilizer N (preferably as a split application).

In the absence of a reasonably significant response to fertilizer N, it is difficult to assess the relative worth of nitrogen fixation to different legume lines. While on examination of the roots of the plant for nodule mass, size, colour (leghaemoglobin) and distribution can give some indication of fixation potential, a measurement of fixation rate may be the only effective means of distinguishing between plants.

In general, methods for measuring nitrogen fixation in the field are difficult to use, time consuming, expensive and in some cases, provide ambiguous information. The following are methods which could be used:-

1. Nitrogen Increment

The nitrogen fixed by a nodulated plant is measured by comparison with a non-nodulated plant and a plant in which yield is maximised by addition of nitrogenous fertilizer. Kjeldanl analysis would usually be used to estimate %N in plant material; however, more novel techniques may become available which are simple, quick and amenable to automation. Plans for field trials of this sort, designed specifically to assess inoculation response may be found in "A Manual for the Practical Study of Root-Nodule Bacteria - IBP Handbook No. 15" by J.M. Vincent (1970).

2. Acetylene Reduction

Most field techniques involve the removal of the root material from the soil and its incubation in an atmosphere containing C_2H_2 (10% $^{v}/_{v}$). Usually C_2H_4 production is measured over a short period following gas chromatographic separation and detection by flame ionisation. While simpler field gas chromatographs utilising a smoke detector have been developed and chemical methods for the selective removal of C_2H_2 designed (thus eliminating chromatography), the assay remains a non-integrative measure of nitrogenose activity performed under relatively sub-optimal conditions and subject to large variation. More recent techniques in which C_2H_2 is injected directly beneath the legume and C_2H_4 formed is measured in the gas mixture diffusing from the soil surface (T.A. Larue, Boyce Thompson Inst., Connell University, personal communication), might however offer a more rapid, simpler and non-destrictive field method.

3. Solute Analysis

While many temperate legumes produce the same translocated solutes of nitrogen, whether they are predominantly fixing N_2 or utilizing combined nitrogen, this is not the case for many tropical species (Pate *et al.* 1980). When N_2 is fixed, the nodules produce the ureides allantoin and allantoic acid which decline as a proportion of total xylem-base N as increasingly

the plant relies on combined N. In the latter case NO_3^- and the amides, asparagine and glutamine, predominate in xylem exudates. The high degree of correlation found between N_2 fixation and ureides in xylem suggested that this might form the basis of a simple field assay. While the use of a xylem sap technique has not been tested widely in the field, studies in which leaf samples of cowpea were tested for ureides in a wide variety of field grown materials indicated that a simple tissue test would not be useful except under highly standardised conditions (E. Pulver. IITA Nigeria, personal communication).

4. ^{15}N Analysis

The use of ^{15}N application in the field to measure fixation both during crop growth and at final harvest has been explored extensively in the two legume-oriented co-ordinated research programmes of the Joint DIVISION of FAO/IAEA, Soils Section and the reader is referred to their reports for details. While this technique is of considerable value in assessing fixation, it is unlikely to be useful on a wide scale in the selection of effective nitrogen fixing symbioses.

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Table 1 Theoretically-based cost estimates for the energy required by component processes in actively fixing legume nodules[1]

Item of functioning	mol glucose/mol N_2
Nitrogenase/Hydrogenase	0.66 - 1.38
Ammonia assimilation and related carbon metabolism[2]	0.14-0.16
Transport of fixed nitrogen	0.13
Growth and maintenance of nodule	0.2 - 0.7
Total	1.13-2.37

[1] The assumptions used in making these estimates are that glucose is respired by oxidative phosphorylation and yields 38 ATP/mol, and that $2e^- = 3ATP$

[2] These values apply to symbioses in which asparagine is the major solute produced as well as those in which ureides, allantoin and allantoic acid, predominate.

TABLE 2

OCCURRENCE OF UREIDES IN XYLEM SAP OF NODULATED LEGUMES

Species in which ureides are major solutes of N in xylem sap	Species in which ureides are minor solutes of N in xylem sap	Species in which analysis has not detected ureides in xylem sap
Albizia lophantha (1)*	Cicer arietinum (14)	Lathyrus cicera (14)
Arachis hypogea (14)	Lens esculenta (14)	Lathyrus sativus (14)
Cajanus cajan (14)	Pisum arvense (12,13)	Lupinus albus (9)
Cyamopsis tetragonoloba (10)	Vicia ervilia (14)	Lupinus angustifolius (14)
Glycine max**(6,7,10,15)	Vicia sativa (14)	Lupinus cosentinii (14)
Macrotyloma uniflorum (10)		Lupinus mutabilis (14)
Phaseolus vulgaris (2,8)		Pisum sativum (4)
Psophocarpus tetragonolobus**(10)		Trifolium repens (3)
Vigna angularis (10)		Vicia calcarata (14)
Vigna radiata (10)		Vicia faba (11)
Vigna mungo (10)		
Vigna triloba (10)		
Vigna unguiculata**(5)		
Vigna umbellata (10)		

* also contains citrulline

** ureides also major solutes of N in xylem sap of detached nodules

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Table 3 C and N Economy of the Root Systems of Nodulated and Non-Nodulated (NO_3 Grown) *Lupinus albus*

Measurements over 10 days in vegetative growth

Item	Nodulated	Non-Nodulated
1. Total plant net photosynthate (m mol C)	205	229
2. % net photosynthate to root system	59	50
3. N assimilated by the root system (m mol N)	6.0	5.5[1]
4. C utilised by root system /N assimilated (mol/mol)	20	21
5. C lost as CO_2 in respiration of root system (m mol C)	69	52
6. C as CO_2 lost/N assimilated (mol/mol)	11.6	9.5

[1] 90% of total N assimilated from NO_3

Table 4 Data Taken from Hardy[1] for Soybean in Field Trials

	Air	Air + 0.15% CO_2
plant N (kg/ha)	295	511
N fixed (kg/ha)	76	427
% N fixed	26	83
Pod Yield (%)	100	160

[1] IV th International Congress of Photosynthesis Reading 1977

Table 5 **Estimates of the plants net photosynthetic resources allocated to nodule functioning during three periods of growth for three symbioses.**

Lupinus albus : Rhizobium WU425[1]

Stage of growth	Vegetative	Flowering and Fruit Set	Pod Filling
Days after Sowing	0-49	50-94	95-135
Total C imported/ N fixed (mg/mg) in nodules	6.5	6.1	7.5
Total C imported by nodules as % of net photosynthesis	31.8	16.9	14.9
Total C imported minus C exported from nodules/N fixed (mg/mg)	4.5	3.9	5.0
Total C utilised in nodule functions as a % of net photosynthesis	21.8	10.9	9.9

Vigna unguiculata : Rhizobium CB756[2]

Days	0-61	62-78	79-120
mg/mg	3.5	2.9	3.5
%	14.8	9.1	4.7
mg/mg	2.3	1.3	2.0
%	9.9	4.0	2.7

Vigna radiata : Rhizobium CB756[3]

Days	0-36	37-58	59-98
mg/mg	8.0	4.2	2.8
%	23.1	10.0	6.3
mg/mg	5.4	2.8	1.4
%	15.6	6.7	3.2

[1] Values derived from data in Pate & Herridge (96); Atkins, Herridge & Pate (9); Layzell et al (68).

[2] Values derived from data in Herridge & Pate (58); Atkins, Herridge & Pate (9); Layzell et al (68).

[3] From unpublished data of R.M. Rainbird, C.A. Atkins and J.S. Pate.

Table 6. Effect of manipulation of leaf area and position at anthesis on the distribution of DRY WEIGHT and overall yield of cowpea plants at maturity.

Manipulated	Roots and Nodules	Stem Petioles and Peduncles	Green Leaves	Senesced Leaves and Petioles	Total Vegetative Yield	Tissue Removed in Manipulation	Total Reproductive Parts	Total Harvested Seed	Total DM Yield
	(g Dry weight/100 plants)								
None	381	318	138	166	1003	-	691	540	1694
$\frac{1}{3}$ leaf area removed	369	247	90	111	817	105	627	502	1549
$\frac{2}{3}$ leaf area removed	429	218	79	73	799	208	464	352	1471
Leaves at nodes 1, 2 and 3 removed	375	221	73	64	733	203	622	454	1390
Leaf at node 4 removed	471	247	108	130	956	97	670	511	1733
Leaves at nodes 4 and 5 removed	319	257	137	71	783	208	593	460	1584

Table 7. Effect of manipulation of leaf area and position at anthesis on the distribution of NITROGEN and overall yield of nitrogen of cowpea plants at maturity.

Manipulated	Roots and Nodules	Stem Petioles and Peduncles	Green Leaves	Senesced Leaves and Petioles	Total Vegetative Yield	Tissue Removed in Manipulation	Total Reproductive Parts	Total Harvested Seed	Total N Yield
	(g N/100 plants)								
None	3.8	4.4	3.6	1.9	13.7	-	21.4	19.8	35.1
$\frac{1}{3}$ leaf area removed	3.2	3.2	2.6	1.3	10.2	2.1	19.9	18.6	32.2
$\frac{2}{3}$ leaf area removed	3.3	2.9	2.7	0.8	9.7	4.3	13.4	12.8	27.4
Leaves at nodes 1, 2 and 3 removed	3.4	2.7	2.5	0.7	9.3	3.0	18.1	16.4	30.4
Leaf at node 4 removed	4.1	3.3	3.0	1.5	11.9	1.8	20.5	18.7	34.2
Leaves at nodes 4 and 5 removed	3.5	3.5	3.8	0.8	11.5	4.0	16.5	15.3	32.0

TABLE 8 PROPOSED STEPS IN RHIZOBIUM-LEGUME SYMBIOSIS[1]

I	PREINFECTION		
	1. Muliplication on root surface ("rhizoplane")	Root colonisation	Roc
	2. Attachment to root surface	Root adhesion	Roa
	3. Branching of root-hairs	Hair branching	Hab
	4. "Marked" curling of root-hairs	Hair curling	Hac
II	INFECTION AND NODULE FORMATION		
	5. Formation of infection thread	Infection	Inf
	6. Development of polyploid (disomatic) meristem; nodule development and differentiation	Nodule initiation	Noi
	7. "Intracellular" release of Rhizobia from infection thread	Bacterial release	Bar
	8. "Intracellular" multiplication of Rhizobia and development of full bacteroid form	Bacteroid development	Bad
III	NODULE FUNCTION		
	9. Reduction of $N_2 \rightarrow NH_4^+$ (Nitrogenase)	Nitrogen fixation	Nif
	10. Complementary biochemical and physiological functions	Complementary functions	Cof
	11. Persistance of nodule function	Nodule persistance	Nop

[1] Proposed by Vincent and others. 6th Australian Legume Nodulation Conference, Perth 1979.

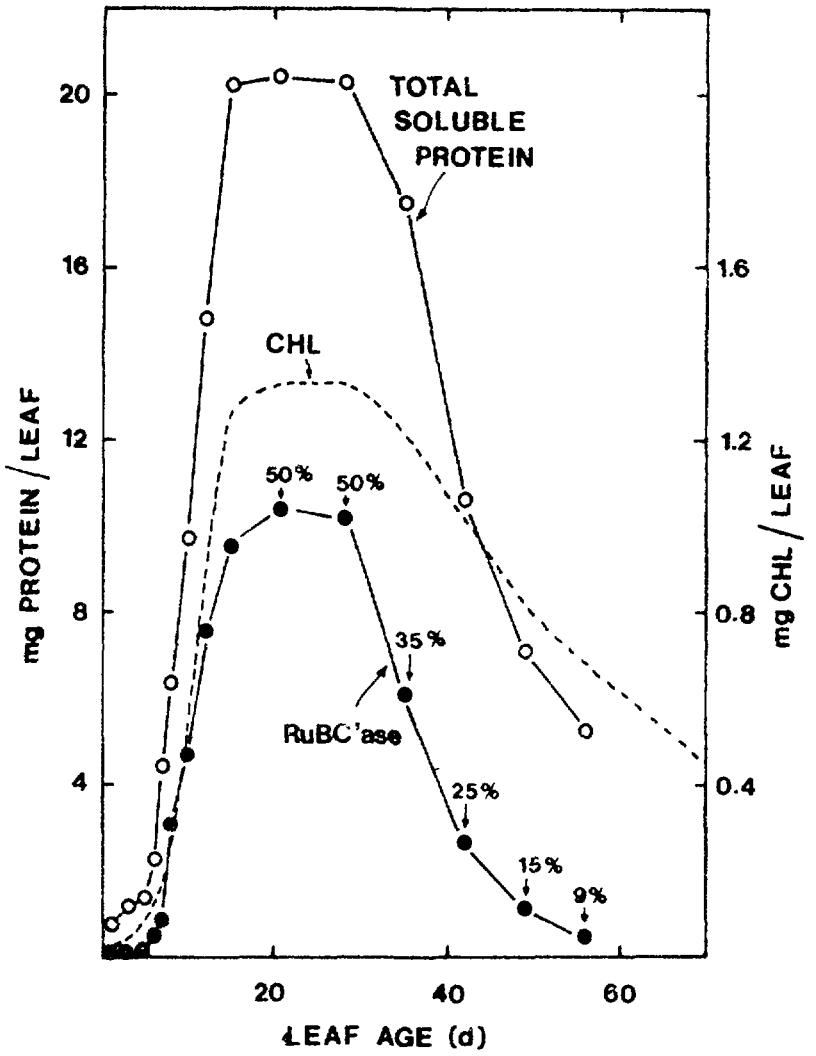


Fig. 1 . Changes in total protein, RUBP C'ase protein (assayed immunochemical ly) and chlorophyll in cowpea leaves during growth and senescence.

CONCLUSIONS AND RECOMMENDATIONS

2nd FAO/IAEA-RCA Research Coordination Meeting on the Use of Induced Mutations for Improvement of Grain Legume Production

Chiang Mai, Thailand, 27 April — 1 May 1981

Following the establishment of the Coordinated Research Programme in 1977, a first research coordination meeting had been held 27 May - 1 June 1979 at Kuala Lumpur, Malaysia. Objectives of the joint programme were formulated and comprised climatic adaptation, productive plant architecture, production physiology, grain quality, resistance to pathogens and to pests of ten different grain legume species relevant as food crops in South East Asia. Now, two years later, reports indicated good progress in many of the objectives and work will continue along these lines for a number of years. Considerable emphasis was placed in the first meeting upon disease resistance, thanks to the participation of another research group concerned primarily with the use of induced mutations for improving disease resistance in grain legumes. During the second meeting, the topic of symbiotic nitrogen fixation was specifically raised, in order to call attention of mutation breeders to the possibility of developing mutant lines with improved nitrogen fixation ability.

Following the presentation and discussion of research progress reports and the consideration of mutation breeding for better nitrogen fixation, the participants agreed to endorse the conclusions and recommendations of the previous meeting (Kuala Lumpur 1979) but considered the following amendments as important:

I. Methodology

A. M_1 Generation: M_1 populations should be large, preferably exceeding 10000 surviving plants. However, if facilities and resources do not allow to handle such populations, the same results may be achieved by smaller experiments repeated for a number of years. At least 1000 surviving M_1 plants per treatment are required.

B. Treatment: To be able to compare results of different experiments, it is essential to ensure more precise dosimetry. The IAEA Laboratory can assist in calibration of dosimeters. A great variation seems to exist regarding chemical mutagen treatments in terms of mutagens, concentrations, treatment conditions, pre- and post-treatments. It is intended to sort out this variation at the next coordination meeting based upon precise description of method used.

C. M2 Generation: Unless all seeds from each surviving M_1 plants are harvested, one should aim at harvesting an equal number per M_1 plant, either randomly or systematically from different plant parts assumed to be derived from independent meristematic regions in the mutagen treated embryo. Whenever possible, progeny-row cultivation should be practiced, as this facilitates the detection of less obvious genetic variation. For progeny rows, at least 20 seeds per M_1 plant would be required. There may be conditions, however (mainly depending upon breeding objectives and screening techniques available), where M_2 bulk populations could be preferred, followed by progeny rows of selected plants in M_3. In general, it is advantageous to have the M_2 space planted.

D. Plant architecture: Mutations for altered plant architecture should be searched for in different parent genotypes or be transfered subsequently into different genetic backgrounds for evaluation . Such evaluation should be performed under different agroclimatic conditions.

E. Yield estimation: In early generations, the yield should be reported in grams per plant. In later generations, results from yield trials should be reported in kg/ha, however, with clear indication of the actual plot size and the number of replications.

F. Quality testing: Quality characters with rel. low heritability like protein content or oil content, should only be tested in advanced true breeding mutant lines that have promising agronomic characteristics. Quality characters with high heritability (like alkaloid or glycoside content) may be selected in early generations.

G. Rhizobial symbiosis for nitrogen fixation:

1. In case of mutation breeding programs aimed at increased legume seed or biomass yield: Some attention should be given to an assessment of the nodulation and nitrogen fixation of the lines selected. While the parent material could be adequate in this respect, mutant lines might not nodulate or might form ineffective symbioses with the natural soil population of Rhizobium. Nodulation can be assessed rapidly in seedling stages by inoculation in glasshouse or field trials (see IBP Handbook No. 15 by J.M. Vincent)[1] but assessment of dry matter or seed yield at final harvest in the field allows a more complete expression of the "effectiveness" of the symbioses. Such an assessment is best made using a soil relatively low in available nitrogen such that the yield of the line as a nodulated crop may be compared with that of the same line grown with a non-limiting amount of fertilizer nitrogen added to the plot. Effective nodulation could require inoculation of seed with strains of Rhizobium not plentiful in the test soil and in such cases the co-operation of a Rhizobiologist should be sought.

2. In case of mutation breeding programs aimed at increasing nitrogen fixation in legumes. Selection of mutants from a large population at the M_2 level would require their comparison with the parent material for this trait. Selection criteria might be for "earliness" of nodulation so that the symbioses become functional as rapidly as possible or for persistent nodulation so that nitrogen input is maintained during pod-filling. The second could be more important if the overall breeding goals include selection for determinate habit. In both cases, soils low in available N should be used to allow maximum expression of the symbioses. Irrespective of the criteria for selection the seed should be inoculated with Rhizobium strains which provide effective nodulation in the parent material.

3. In case of breeding programs aimed at the selection of Rhizobium strains for increased nitrogen fixation: A number of desirable traits for nodulation in particular crops under particular conditions may be recognised. These are high or low temperature tolerance, tolerance to acid soil conditions, competitiveness in soil under normal agricultural practices, resistance to desiccation and broadened host specificity (virulence).

1) J.M. Vincent: A Manual for the Practical Study of Root-Nodule Bacteria. Blackwell Scient. Publ. Oxford 1970.

H. Use of mutants in cross breeding: It appears highly desirable to cross selected mutants with each other, with the parent cultivar, and with unrelated genotypes to widen genetic variation and allow for recombination.

I. Cooperative trials: Testing of mutant lines in different countries will help in evaluating improved productivity. The exchange of mutant material among cooperators is recommended, subject to application of quarantine rules.

II. Crop Plant Species

A. Soybean (Glycine max)

1. Yield: Most important characters to be improved would be number of pods/plant and number of seeds/pod. There appears to be a negative correlation between seed size and total yield.

2. Plant architecture: It is generally assumed that soybean has more leaf area than is required for good grain yields. There are claims that narrow and longer leaves will provide better yields. For such types, the plant density/unit area might be increased. This would also apply for short stature types. Farmers need better lodging resistance and would prefer rather determinate types. Pods should not develop too close to the ground in case of mechanical harvesting.

3. Adaptation: Soybean is often too sensitive to day-length and temperatures. Adaptation to high altitude and high latitude cultivation would require mutations for less sensitivity to these two factors. Early maturing types are also advantageous for other conditions.

4. Resistance: Important would be mutants with improved resistance to rust, soybean mosaic virus, Cercospora leaf spot and to the bean fly (Agromyza sp).

Other characters: High oil and protein content must be given considerable attention. Seed viability should also be considered. Of great value would be mutants that are not shattering the seeds when harvest is delayed by a few days.

B. Peanut (Arachis hypogaea L.)

1. Yield: Improvement of various yield components, harvest index and shelling percentage should be aimed at.

2. Plant architecture: Efforts to modify plant architecture should continue. A good example is the success in developing a spreading plant type with maximal pod setting through sequential flowering (as in the "Spanish" varieties) by Patil, India. This type shows less crop loss at harvesting due to stronger pegs.

3. Adaptation: In order to fit peanuts into different growing seasons and cropping systems, mutants with altered growth period but the same yield potential as current commercial varieties would be of value.

4. Resistance: Sources of resistance to Cercospora leafspot, rust, rosette virus and Aspergillus flavus are very limited.

5. Seed characters: Peanuts intended for direct consumption require good pod and kernel appearance and should have a high protein content. For industrial processing, mutants with higher oil content while maintaining present fatty acid composition would be desirable. Seed dormancy is desired by farmers, but the dormancy period should be short.

C. Common bean (Phaseolus vulgaris)

1. Improvement of yield components, early flowering and rapid pod development should be aimed at. High symbiotic nitrogen fixation efficiency should be looked into.

2. Plant architecture: Mutation induction would be suitable to alter adapted high yielding winding types into bushy determinate types, to be cultivated without the need for staking.

3. Resistance: Induced mutations would be desirable for improved resistance to important diseases such as anthracnose (Colletotrichum lindemuthianum) root rot (Fusarium oxysporum), bean rust (Uromyces sp), and mosaic disease.

4. Grain quality: The nutritional quality (protein) as well as the eating quality (cooking time, flatulence) could be improved.

D. Mungbean (Vigna radiata)

1. Yield: The yield may be increased by restructuring the plant so as to increase number of branches, number of clusters, number of seeds/pod, number of seeds and seed size. Important would also be a more synchronous maturity and less shattering.

2..Adaptation: Increasing daylength tolerance would allow the expansion of cultivation into different seasons and locations.

3. Resistance: Improvements would be very desirable for resistance to Cercospora leaf spot, mungbean yellow mosaic virus, powdery mildew (mainly in Bangladesh) and mungbean rust, but also against aphids and bean fly.

E. Pigeon pea (Cajanus cajan)

1. Yield: Higher grain productivity would require alteration of the plant type and improved partitioning of photosynthates to the grain. After careful consideration of specific management, cropping and agroclimatic conditions, determinate growth habit and suitable crop duration might be introduced into the new plant type.

2. Resistance: Pod borer (Heliothis armigera) and pod fly (Melanogromyza obtusa) cause severe damage to the crop. No resistance is available in germ plasms, therefore, mutation induction should be a useful approach. Fusarium wilt and sterility virus are major diseases of pigeon pea, however, resistance sources are available and cross breeding for transferring the resistance would be appropriate.

3. Grain quality: Like in other grain legumes, sulfur containing aminoacids are limiting the biological value of the protein. Natural variability for these amino acids is reported to be very narrow. Mutation breeding might be a useful tool to enlarge genetic variability for these amino acids.

F. Chick pea (Cicer arietinum)

1. Plant architecture: Most desirable would be mutants with erect and short stature, and early maturing for high input conditions (Irrigation, fertilizer).

2. Resistance: Required would be resistance against wilt, root rot and blight. Damaging insect pests are mainly Heliothis punctifera and H. armigera. Other pests are of regional or local importance.

3. Grain quality: While improving grain yield, quality characters have to be monitored (protein).

G. Black gram (Vigna mungo)

1. Climatic adaptation: For allowing year round planting, daylength (seasonal) neutrality would be desirable. Short duration varieties would fit easier into various crop rotations and might reduce the risk of misharvest. In this context, improvement of drought tolerance, and to a certain extent, cold tolerance, would also be important objectives.

2. Plant architecture: Erect and bushy types, with more branches and more clusters of pods promise higher grain yields. Uniformity in flowering, pod setting and maturity would reduce yield losses and make harvesting easier.

3. Grain quality: Large and uniform seed size and dark colored big hilum are requested by the market for making bean sprout. Protein content should be monitored.

4. Resistance: Sclerotia, collar rots, Cercospora leaf spot, yellow mosaic virus and powdery mildew are important diseases, bean flies are a damaging pest.

H. Pea (Pisum sativum)

1. Plant type: Varieties suitable for high input conditions should be short and have better lodging resistance.

2. Resistance: There is a need for additional sources of resistance against powdery mildew and a number of diseases of local importance. Resistance against pod borers would be desirable.

I. Lentil (Lens esculenta)

1. Mutagen response: As mutation research with lentil has been extremely limited, more experience is necessary with regard to effectiveness of various mutagens and the extent of induced variability that can be expected.

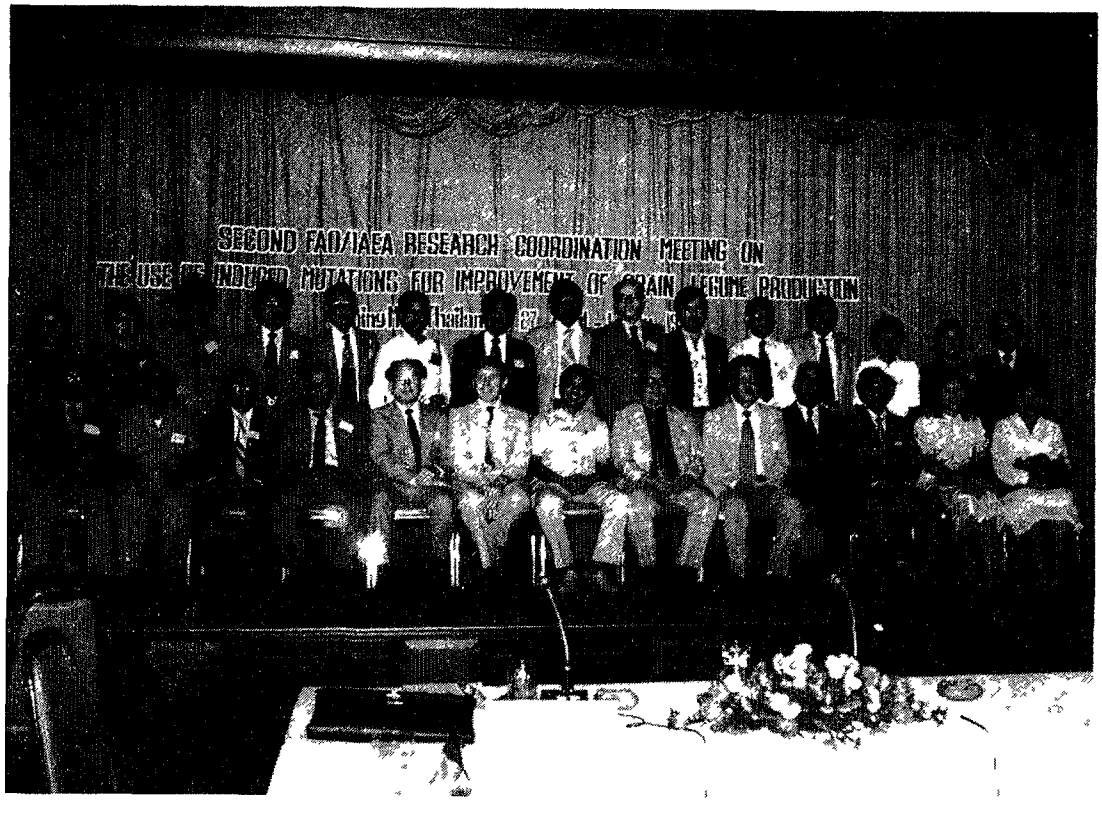
2. Yield: Plant types with high grain production, responsive to inputs of fertilizer and water are sought. Earliness and daylength-tolerance are desirable in the macrosperma lentils.

3. Resistance: Valuable would be improved resistance against wilt, rust, blight, aphids and pod borers.

J. Faba bean (Vicia faba)

This species is not only important in the Mediterranean region but is also gaining interest in South East Asia. Resistance against diseases and pests will be crucial for good productivity. Orobanche crenata is a very destructive parasite in the Mediterranean region, and as there is no satisfactory resistance, mutation breeding is the great hope.

Opening of the meeting: Deputy Governor of Chiang Mai Province (middle), Deputy Director General, Department of Agriculture (right) and Scientific Secretary addressing the participants.



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