Seminar on nuclear techniques in agriculture and environment

Kuala Lumpur, Malaysia

8 - 11 June 1981

USE OF INDUCED MUTATIONS IN SOYBEAN BREEDING

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ABSTRACT

Artificial induction of mutation in plants is carried out using y-irradiation and ethyl methanesulphonate (EMS) to expand the genetic variability of locally-grown soybean. This aspect of mutation breeding complements the conventional breeding approach undertaken by the Joint Malaysia Soybean Breeding Project group. Recovery of agronomically-important mutants such as earliness, lateness, bigger seed size and improved plant architecture were recorded. The significance of these findings is discussed.

INTRODUCTION

Mutation, whether spontaneous or induced, has a function of generating and expanding the germplasmic base of a given crop species. The former process is very conservative and occurs very rarely in nature. However, it provides the only way for generating raw materials upon which selection can act.

Mutation induced through artificial means is no different from that occuring spontaneously. According to Gaul (2) not only the whole spectrum of genotypes known in nature could be reconstituted artificially, but novel genotypes could also be induced.

Attempts to use induced mutations for soybean breeding in Malaysia were prompted by several factors. Chief among them was the fact that commercial planting of soybean had never been viable in Malaysia due to lack of locallyadapted varieties. Secondly, in 1978 a major initiative involving five different local institutions was started to undertake the task of breeding the soybean to the lowland tropics of Malaysia. This set-up is known as the Joint Malaysia Soybean Breeding Project (JMSBP) (4). For the most part it involves conventional breeding methods, but mutation breeding remains an important supplementary technique. Thirdly, induced mutations had been tried out in Malaysia and found to be promising to some extent (3) (1).

OBJECTIVES IN MUTATION BREEDING OF SOYBEAN

Of the five partners in the JMSBP, only the Rubber Research Institute of Malaysia (RRIM) and Universiti Kebangsaan Malaysia (UKM) are directly involved in the induction and early testing of mutants. The other institutes would be involved in conventional breeding and also the evaluation of advanced materials from the mutation breeding project. General objectives of the programme fall into two broad areas, namely the breeding of local varieties to be grown as an intercrop in immature rubber and oil palm holdings, and also as a rotational crop with rice or other annuals. Specifically, the programme sought to alter the plant architecture sufficiently so as to result in an improved 'sink capacity' in the form of higher number of pods per plant, higher number of seeds and bigger seeds. Selection for mutants resistance to pests and diseases is also carried out in addition to seed coat colours which may be preferred by consumers. The last but not least important an objective is the induction of mutants with altered flowering or maturity period. In the humid tropics particularly Malaysia, where the soybean is only of recent origin (6). The plant tends to flower prematurely resulting in stunted height, poor branching and low pod set. Consequently, seed yield is reduced significantly. To a large extent poor adaptation to the local environment can be attributed to this adverse factor.

SOURCE OF MUTATIONS

Mutations in plants can be induced by ionizing radiation such as x-rays, gamma rays, neutrons, or several other physical mutagens, which in general result in major chromosomal aberrations. In addition, chemical mutagens such as nitroso compounds, sodium azide and ethyl methanesulphonate (EMS) have produced satisfactory level of mutagenesis in crop plants. Chemical mutagens are more specific in their actions and result in mutations at individual gene loci (5).

In our series of experiments, two sources of mutagens are used, namely gamma rays from a cobalt-60 source located at the Nuclear Science Unit of Universiti Kebangsaan Malaysia, and a chemical mutagen, EMS obtained from Sigma. The detailed procedures of the experiments have been described elsewhere by Zakri **et al.** (7). Basically it involves the exposure of dry seeds (ca. 12% moisture content) to selected doses of gamma irradiation, corresponding to specific level of survival values in the M_1 generation. In soybean the range may vary from 5 kr to 25 kr. In the EMS experiments, a strict regime of presoaking the seeds for several hours before treatment, treatment proper of approximately 3 hours in EMS concentration ranging from 1.0 to 2.5%, followed by a postwashing period in the region of 18 hours, may be enforced to ensure reproducibility of results. Seeds that have been treated to either gamma rays or EMS are normally immediately planted in the field.

SOURCE OF MATERIALS

Soybean seeds used in the mutation experiments are usually derived from foreign introductions which have been found to be fairly well-adapted to local conditions. Two soybean entries that are widely used as parental materials in our work are **Acadian** and **Palmetto**.

To recover mutants in reasonable numbers, a large population size is preferred even though this may pose some problems of management. In our experiments it is not uncommon to use 40,000 seeds per treatment per variety with 10,000 seeds per experiment for control.

FIELD HANDLING

To accomodate for the expected low percentage of survivals in the M_1 generation and to avoid excessive branching and hence preventing the occurence of diplontic selection, a closer spacing among plants is practised. If the normal spacing is 50 x 10cm, the treated materials are usually sown at 40 x 5cm (for low dosages) or at 40 x 2cm (for high dosages). In the M_1 generation, data on germination, survival and fertility are recorded.

No selection for mutant is done in the M_1 due to the chimaeric nature of the individuals. Seeds harvested at maturity to make up the M_2 population are sampled from two to four pods taken at random from different parts of each M_1 plant and bulked as per treatment and variety. Ideally one would have preferred to derive

only one seed per plant to avoid duplication of mutation type occuring in the same individual, but practical constrait ts normally do not allow for this.

In the M_2 generation (Fig. 1) seeds are spaced-planted at 50 x 10cm, and observation and selection of plants with mutant traits are started during this time. Individual plants are tagged and at maturity they are harvested separately.

Seeds from individual M_2 plants are planted in rows in the next generation (M_3). Traits for which they are earlier does not appear to have a genetic component, the individuals selected would be discarded. If it is confirmend, progeny lines would be perpetuated in advanced generations for testing by other cooperators in the main soybean programme. Lines selected would either be released directly as new varieties or used as parents in conventional breeding programmes.

RECOVERY OF MUTANTS

Table 1 presents some mutant characteristics recovered from one of our experiments (7) The fine traits are earliness, lateness, seed coat colour, seed size and gigas morphology (Figs. 2 to 5). In characters like earliness and seed coat colour, there is evidently a varietal influence on the action of mutagen. Palmetto appears to be more sensitive to mutation induction than Acadian. However for characters such as lateness and seed size no marked difference is observed in mutant frequency among the two varieties. Two gigas plants with a frequency of 1.8×10^{-5} are recovered from the mutagen-treated Palmetto. The gigas proves to be an interesting phenotype. It is more robust in appearance, with a distinct increase in height and other morphological traits. The gigas individual is also characterized by a delayed time of maturity. Further tests on the agronomic potential of this phenotype and its genetic nature are currently being carried out.

With respect to the two types of mutagens employed, there is some apparent difference in the mutant frequency induced but it is still too early to make any definite conclusion on their varying effects. On the whole it appears that EMS tend to be more effective than gamma rays (Table 1).

A general comparison on the mutation rate and the mutant frequency of some traits recovered is given in Table 2(7). It is observed that the induction of altered maturity period, whether it is for earliness (1.3×10^{-3}) or lateness (5.4×10^{-3}) is relatively to achieve. According to Micke (1970 induced mutation techniques can be applied towards this objective with a good chance of success. The induction of seed coat colour change may have some significance in soybean breeding in view of consumer preference. From our data it is found that this trait can be induced relatively easy. Seed size as an inherited trait could also be genetically altered ($4.5 - 10^{-4}$) to suit the breeder's objective. In some cases, bigger-sized seeds may be an added advantage.

Tables 3 and 4 offer some indication on the range of values that could be obtained in early and late-maturing soybean mutants respectively. As a general rule, earliness is defined as those that mature 7 - 12 days earlier than the control, while lateness are those that mature 7 - 12 days later than the control. For the earliness mutants (Table 3), among the ten random mutant isolates the range in plant height varies from 16.3cm to 39.0cm, an increase of slightly more than two-fold. Among the yield components, branches per plant can be strongly affected as evidenced from the range of monobranching up to 8 branches/plant. Pods per plant are also proned to change, with a value ranging from 4 to 28. Seeds per pod appear to be the least affected by mutation induction and a range of 2 to 4 is observed. As is evident earlier, seed size, especially bigger seeds, could be recovered with ease. However, all things considered, the early-maturing phenotypes tend to be generally poor yielders. This is mainly due to the low number of pods established on each plant.

For a clearer picture a comparison should be made with Table 4. The lateness mutants are approximately at par with the control with respect to the number of branches per plant and seeds per pod. However, the mutants are really way ahead of the control in terms of pods per plant and seed size. Mutant isolate P630-2 for example, have 86 pods per plant compared to 22 for the control, an increase of four-fold, inspite of the

short stature (18.0cm) of the former. This compact mutant is also endowed with sufficient branching, a slight increase in number of seeds per pod and fairly bigger-sized seeds. In the humid tropics where no marked change of climate exists, breeding for lateness should seem a worthwhile objective to pursue. Prolonging the vegetative phase of the soybean plant would allow for the development of a bigger sink capacity in terms of the yield components. In addition, the period from flowering to maturity should also be long enough for seed filling to take place adequately.

CONCLUSIONS

The use of induced mutations to augment conventional breeding methodology should yield substantial dividends, if objectives are clearly defined at the outset of the breeding programme. Induced mutations can expand the genetic diversity of a crop species and thus facilitate the recovery of agronomically-important phenotypes with better yielding ability. The prospect of utilising such techniques in soybean improvement is demonstrated in the above discussion.

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Table 1. Varietal and mutagen effects on mutant frequency (m) of soybean (after Zakri et al., 1981)

Mutant character	Variety		Mutagen	
	Palmetto (m)	Acadian (m)	EMS (m)	Gammma rays (m)
1. *Earliness*	1.3×10^{-3}	9.7×10^{-4}	3.5×10^{-4}	1.8×10^{-5}
2. *Lateness*	5.5×10^{-5}	5.1×10^{-5}	4.9×10^{-5}	5.7×10^{-5}
3. *Seed coat colour*				
(a) pale yellow	1.6×10^{-4}	-	3.5×10^{-4}	-
(b) black spotted	4.6×10^{-5}	-	1.0×10^{-4}	-
(c) brown spotted	9.1×10^{-4}	-	-	1.1×10^{-5}
4. *100-seed weight*				
> 15 gm	4.6×10^{-4}	4.3×10^{-4}	4.6×10^{-4}	4.5×10^{-4}
5. *Gigas plant*	1.8×10^{-5}	-	2.1×10^{-5}	1.1×10^{-5}

Table 2. Mutation rate and Mutant frequency of induced characters in soybean (after Zakri et al., 1981)

Mutant character	No. Mutants	Mutant Frequency (m)	Mutation rate (m)
1. *Earliness*	173	1.3×10^{-5}	6.3×10^{-5}
2. *Lateness*	747	5.4×10^{-3}	2.7×10^{-2}
3. *Seed coat colour*			
(a) pale yellow	17	1.2×10^{-4}	6.2×10^{-4}
(b) black spotted	5	3.6×10^{-5}	1.8×10^{-4}
(c) brown spotted	1	7.3×10^{-6}	3.6×10^{-5}
4. *100-seed weight*			
>15 gm	62	4.5×10^{-4}	2.2×10^{-3}
5. *Gigas plant*	2	1.5×10^{-5}	7.3×10^{-5}

FIG. 1. M Generation of Soybean

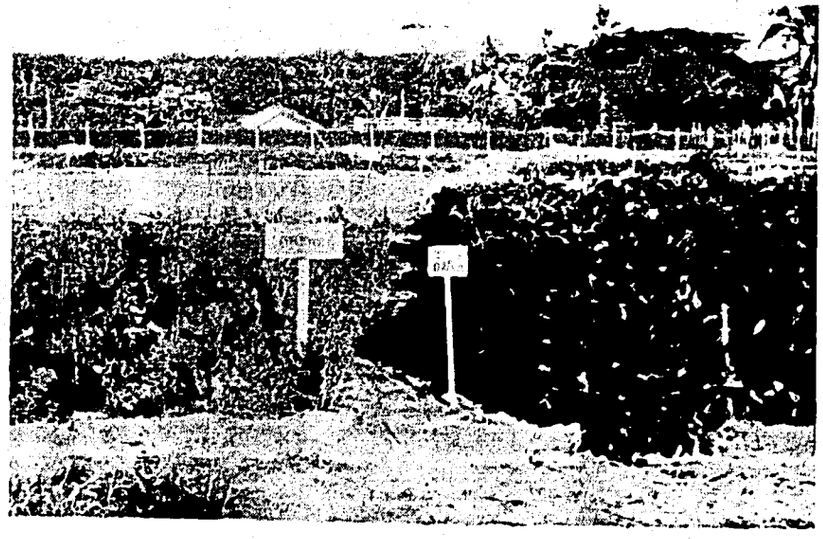

FIG. 2. The Soybean stand on the right comprises of "Lateness" mutants.

Table 4. Yield components of late mutants of soybean cv. palmetto induced by EMS

Mutant isolatates	Plant height (cm)	Branches/ plant	Pods/ plant	Seeds/ pod	100-seed weight (gm)

Table 4. Yield components of late mutants of soybean cv. palmetto induced by EMS

Mutant isolatates	Plant height (cm)	Branches/ plant	Pods/ plant	Seeds/ pod	100-seed weight (gm)
P630-1	41.5	4	73	2.6	17.80
P630-2	18.0	5	86	2.4	17.50
P630-4	46.0	5	76	2.7	16.20
P630-5	45.5	4	62	2.8	17.90
P630-6	37.0	5	63	2.4	14.10
P630-7	48.0	4	51	2.4	18.80
P630-10	40.0	2	48	2.7	17.30
P630-11	42.0	3	40	2.8	19.80
P630-15	25.0	2	45	3.0	13.50
P630-17	27.0	4	30	2.3	19.50
CONTROL	27.6	4	22	2.2	12.01

Table 3. Yield components of early mutants of soybean cv. Palmetto induced by EMS

Mutant isolates	Plant height (cm)	Branches/ plant	Pods/ plant	Seeds/ pod	100-seed weight (gm)
P636-1	35.0	4	31	2.3	13.80
P636-2	24.0	2	10	2.6	10.17
P636-7	37.7	4	23	2.1	19.05
P636-16	19.5	4	23	3.2	15.50
P636-23	36.7	6	28	4.1	16.37
P6324-10	22.6	3	12	2.5	18.00
P6324-33	16.3	1	4	2.0	13.20
P2436-6	39.0	8	29	3.4	10.00
P2436-12	26.2	6	27	3.1	15.10
P2436-23	23.7	3	8	3.4	14.00
CONTROL	27.6	4	22	2.2	12.01

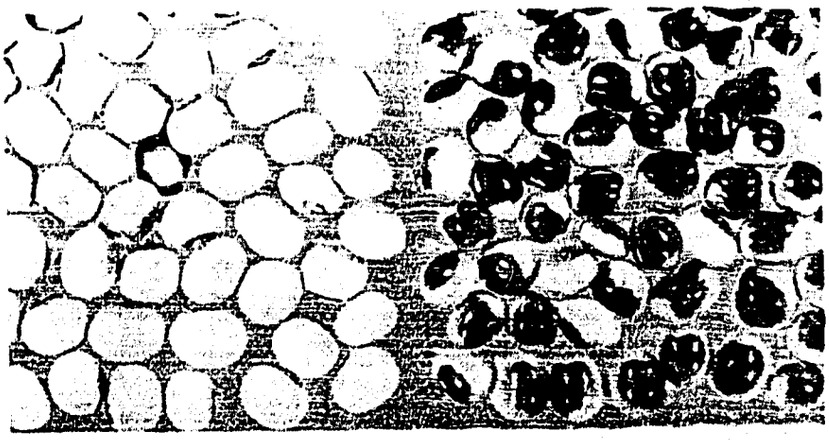

FIG. 3. Line 45 is obtained throngh induced mutation of the mother line, "Palmetto".

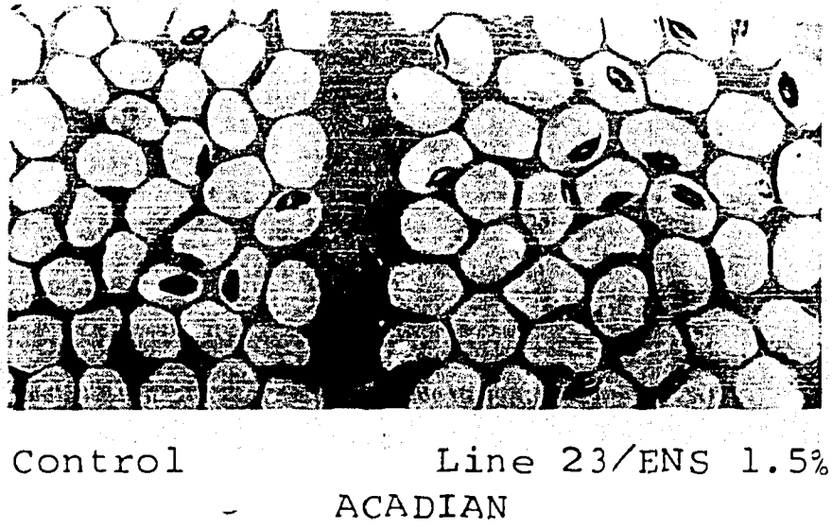

FIG. 4. The seed batch on the right is a bigger size mutant derined from the control.

FIG. 5. The 'gigas' mutant obtained from the mother line, "Palmetto".

DISCUSSION:

Nama: Ramli Abdullah, UM.

Soalan: Can we apply the mutation technique in animal breeding programme.

Jawapan: Most mutations are lethal or semi-letnal. It is not practical to use the technique in breeding of domesticated animals.

Nama: A. Abdul Aziz, RRIM.

Soalan: Do you get the same mutants when another batch of soybean is given a similar radiation dosage.

Jawapan: If the population size used is large enough the same spectrum of mutation types can be possibly recovered.

Nama: H.A. Md. Soot, UKM.

Soalan: You have mentioned several ionising radiation are possible to be used. As far as know, the raizing radiation sources have different energy, will the source affect the work above?

Jawapan: Each type of ionizing radiation would have a different effect on different organism. It is up to the researcher to carry out preliminary trials to evaluate the dose effect of each radiation and make the necessary adjustment.

Nama: N. Poedujono, UKM.

Soalan: Did you analyse the protein contain of amino acid composition.

Jawapan: At present our data on the range of protein conttent in our treated materials are incomplete. We are currently analyzing the data on this trait.