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MUTAGENIC RESPONSE OF PEANUT (*ARACHIS HYPOGAEA* L.) TO FAST NEUTRONS

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ABSTRACT

Dormant seeds of peanut were treated with varying doses of fast neutrons employing efficient pre- and post-irradiation techniques for determination of various plant responses useful in a long range program of mutation breeding. Mean reductions in seedling height and frequency of M_1 somatic mutations increased with increasing radiation dose. No reductions in M_1 seedset was obtained even in the dose above the LD_{50} of 1600 rads. The frequency of M_2 macromutations ranged from only 3.60-5.65 per 100 M_2 plants. The genetic basis of each of these radiation responses is briefly discussed.

The high sensitivity of M_1 peanut seedlings and growing plants to fast neutrons is probably due to the highly differentiated seed embryo at the time of irradiation while the radio-resistance exhibited by the matured plant appears to be related to the polyploid genome of the species.

Introduction

The more widely known human achievements in the utilization of the energy of the atom lies ironically in the field of weaponry for mass destruction. Relatively unknown to the bigger portion of the human population is the use of this energy for the improvement of crops which are of benefit to man. For instance in 1974, about a hundred new crop varieties were listed as having been improved through mutation breeding and officially released by various governments for widescale cultivation. Not a few plant radiation investigators, however, attach greater significance to this human effort than even the socio-economic impact of the improved crop types on the lives of millions of people in the food-hungry world. The emergence of a new technology for inducing hereditary change has revived interest in some of the age-old problems concerning the origin and future of the species, topics that have challenged the minds of thinkers since olden times.

As the pioneering studies of scores of plant radiation workers during the past four decades have not fully explored the intricate problems related to the control and direction of the induced mutation process, there is a need for further investigation for gaining a better mutagenic treatment. Moreover, most of the work in mutation induction through seed irradiation have made use mainly of sparsely-ionizing radiations, such as X-rays and gamma rays, and only a few studies have

dealt with densely-ionizing radiations due more or less to the inavailability of sources of the latter. In the late 1960's, the International Atomic Energy Agency undertook the design and construction of the standard neutron irradiation facility or SNIF, a shielding device for treatment of biological materials with fast neutrons in conventional atomic reactors. As there are still only a few of this type of irradiation facility in existence today, very little work is still available on the genetic effects of fast neutrons on seeds.

The aim of this study was to determine the mutagenic response of peanut, a polyploid, to fast neutrons employing efficient pre- and post-irradiation techniques.

Materials and Methods

Dormant seeds of peanut were stored in a moist dessicator for seven days to bring their moisture content to 14% and then placed in plastic bags for irradiation in the SNIF at the Philippine Atomic Reactor (PRR-1) at doses of 500-2000 rads fast neutrons according to suggested procedures (Konzak and Sigurbjornsson, 1971). Untreated seeds from the same source were used as control. Soon after treatment, the seeds were rehydrated in water for two hours with a constant temperature of 30°C, sown on moist blotting paper in petri dishes and then planted in the field dose-to-row with three replications in Pulilan, Bulacan in September to December, 1983. Some of the seeds were sown in a "blotter sandwich" and the seedlings grown for 20 days in a humid growth chamber with a temperature ranging from 28° – 30°C and continuous flourescent illumination of approximately 120 foot candles for seedling height measurement.

The number of M_1 plants with chimeral sectors was obtained throughout the different growth stages. Seedset was determined from M_1 plants harvested at random in the field. The M_2 seeds were planted in bulk by dose in the field at the Botany Experimental Garden about three months after harvest for determination of the types and frequency of macromutations in the M_2 generation.

Results and Discussion

Data on the seedling height response of peanut seed embryo to varying doses of fast neutrons are shown in Table 1. In general, the reductions in seedling growth as compared with the control more or less increased with increasing radiation dose. Marked mean seedling growth reduction was observed at a dose of 200 rads. LD_{50} for seedling height was found at a dose of approximately 1600 rads fast neutrons. Seedling height response has been widely used as an early indicator of the degree of genetic effects of radiation on seeds considering that reductions in growth of seedlings and chromosomal breakage are highly correlated (Conger and Constantin, 1979). Depression of height of seedlings has been found to occur only when approximately 25-30% of the cells bear chromosomal changes. (Hendry and Howard,

Table 1. M_1 seedling height response of peanut to fast neutrons.

Fast neutron dose	*Total seedlings*	*Seedling height (cm.)*		
		Range	*Mean*	*% Control*
0 (Control)	150	14.5–17.8	15.62	100.00
500 rads	150	13.4–15.7	14.55	93.15
1000 "	150	10.2–13.4	11.36	79.13
1500 "	150	6.7–11.6	8.14	58.52
2000 "	150	3.7– 7.9	5.98	36.28

Table 2. Frequency of M_1 peanut plants bearing somatic mutations after fast neutron seed irradiation.

Fast neutron dose	*Total M_1 Plants*	*Plants with somatic mutations*	
		No.	*Frequency per 100 plants*
0 (Control)	1416	0	–
500 rads	1405	19	13.52
1000 "	1372	144	104.80
1500 "	1398	302	216.02
2000 "	1053	487	462.49

1978). Oxygen contamination during irradiation could result in an interaction between oxygen and radiation-induced oxygen-reactive sites prior to the initiation of soaking (Conger and Constantin, 1979). The advantage of employing neutrons in practical mutation breeding, i.e., reduced oxygen effect, is now widely recognized (Ramulus and Ranjasumy, 1972). Neutrons have been reported to be up to 40 times more effective than sparsely-ionizing radiations on dormant seeds at the D_{50} level of damage but only 6-8 times more effective on germinating seeds (Conger *et al.*, 1973).

The frequency of M_1 plants with somatic mutations which consisted mainly of leaf flecking on seedlings and leaf sectoring (Table 2) increased with increasing fast neutron dose. The formation of somatic mutation in plants growing from mutagen-treated seeds is believed due mainly to chromosomal change. Breakage of chromosomes accompanied by genetic deletion has been found to be the most probable mechanism for the formation of chimeral sectors (Mericle and Mericle, 1967). The high frequency of somatic mutations in M_1 plants after fast neutron seed-irradiation is probably an indication of the effectivity of densely ionizing radiations on biological material when their effects are not altered or modified by

Table 3. Mean M_1 seedset in peanut after fast neutron seed irradiation.

Fast neutron dose	*Total M_1 plants*	*Range (%)*	*Mean (%)*	*% control*
0 (Control)	25	85.76–97.36	91.46	100.00
500 rads	25	73.82–94.14	84.22	92.08
1000 "	25	69.67–88.39	79.65	87.09
1500 "	25	64.85–81.76	72.84	79.64
2000 "	25	51.08–82.35	68.07	74.43

conditions in the cellular environment. Of interest is the suggestion that chimerism often results from a mutation in a few apical cells and will likely become perpetuated in the meristem cells bearing chromosomal aberrations (Conger *et al.*, (1973). Compared with the seedling growth response of barley *(Hordeum vulgare* L.), a species widely used as the standard test material in seed irradiation with an LD_{50} at a dose of 1120 rads (Soriano *et al.*, 1971), peanut is much more radioresistant due invariably to its being a polyploid with a chromosome number of 2N=40 (Ashri, 1982; Kirti *et al.*, 1982). With mungbean (*Vigna radiata*), LD_{50} for seedling height was found close to a thermal neutron dose of 30 x 10^{12th} N/Cm^2/sec (Kwon and Oh, 1983).

In the present study, pre- and post-irradiation conditions known to influence seed response to densely-ionizing radiations (Conger and Constantin, 1979) were largely minimized. Previous studies on peanut even with sparsely-ionizing radiations appear to have failed to consider this aspect of the work except a report (Gregory, 1968) dealing with gamma ray treatment of seeds with only 8% moisture content.

Modification of the effects of fast neutrons with seed moisture content and oxygen have definitely been achieved (Angstrom, 1968) contrary to a previous idea that only those of sparsely ionizing radiations could be so modified. However, seed moisture content has been reported to be the most important factor modifying fast neutron effects (Conger and Carabia, 1972). Post-irradiation storage likewise has been found to enhance fast neutron damage (Gopal-Ayengar *et al.*, 1977). Lower reductions in OER were reportedly obtained with increasing ionization density for as long as such mutated cells retain their apical position (Balkenia, 1972). Chimerism in plants with irradiated origin has been specifically associated with persistent dicentric chromosomes (Contant *et al.*, 1971). Furthermore, leaf sectoring after exposure to radiation was reported to be due to chromosomal aberrations which increase exponentially with dose (Kaplan, 1977).

The data on M_1 seedset are shown in Table 3. No significant reduction in ovule fertility after fast neutron seed irradiation was found even in the dose higher than LD_{50}. This radioresistance may be due to the polyploid nature of the peanut

Table 4. Frequency of macromutations in some M_2 lines of peanut after fast neutron seed irradiation.

M_2 line	*Total plants*	*No. of macromutants*	*Frequency of macromutants per 100 M_2 plants*
8446– 1	194	11	5.65
8446– 2	259	9	3.60
8446– 3	197	8	4.06
8446– 4	236	10	4.24
8446– 8	608	23	3.78
8446–14	243	12	4.94

genome (Ashri, 1983). While the M_1 seedlings and growing M_1 plants showed high sensitivity to radiation, the mature plant failed to manifest a similar degree of response. Polyploids are known to be quite resistant to mutagenic treatments due probably to their having more than a duplicate set of chromosomes. At the molecular level, polyploids have been reported to contain more DNA per cell than their diploid counterparts (Sparrow *et al.*, 1971) and are thus able to overcome the effects of genetic damage effectively.

Table 4 shows the frequency of macromutants in the M_2 generation. These consist mainly of dwarf plants, plants with five-leaflet leaves, odd-pinnate leaves, variegations, dark-green plants and early flowering, characters previously found to be inherited in the selfed progeny of the M_2 variants. The frequencies of macromutants of 3.60-5.65 per 100 M_2 plants in the six M_2 lines are rather low compared with mutation frequencies in diploid species after seed treatment with either sparsely- or densely-ionizing radiations. Both types of radiation reportedly induce the same types of genetic changes (Borojevic, 1975). Compared with neutron-irradiated material, the germinal mutation frequencies obtained in peanut are rather low. For instance in soybean, an M_2 mutation frequency of 24 per 1000 M_2 plants was obtained after irradiation of seeds with a dose of only 1000 rads fast neutrons (Hendratno *et al.*, 1982) and in mungbean, mutation frequencies of 8.43% and 10.76% were obtained after seed treatment with thermal neutrons (Kwon and Oh, 1983).

Most of the mutant types were, however, partially sterile due probably to chromosomal damage possibly of the exchange type which reportedly could get transmitted to the succeeding generations (Schori and Ashri, 1970). The association between a mutant character and chromosomal aberration may explain the low mutagenic specificity commonly obtained after seed irradiation (Smith, 1972). Fast neutrons have been reported to be more effective than sparsely ionizing radiations for inducing genetic variations which may, however, be mostly quantitatively in-

herited, an indication that those mutations are due to chromosomal alterations rather than "point" mutations (Daly, 1973).

Finally, the genetic response of peanut at the mature stage appears typical of polyploids characterized by low response to treatment. This is evidenced by the relatively small reductions in seedset and low frequency of M_2 mutations. This low response of peanut to fast neutrons is most probably due to the tetraploid genome and the somatic competition that invariably occurs between normal and mutated cells during the growth stages of the M_1 plant. The chromosome number of peanut of 2N=40 may have originated either through polyploidization of a wild species (*Arachis monticola*) with 2N=20 or through fusion of the diploid genomes of *A. villosa* which is believed to have contributed the A-genome of cultivated peanut and *A. baticoci*, the B-genome (Kirti *et al.*, 1982). On the other hand, the high sensitivity of peanut seedlings and growing plants may have been brought about by its well-differentiated seed embryo (Emery, 1972) with an extremely large plumule which consists of 10 leaves and four axillary buds. As the epicotyl does not produce new parts during the first three weeks of growth, radiation damage in the treated seed embryo is reflected by the high response of the M_1 seedlings of that age.

Summary and Conclusion

1. In general, a linear dose-response relationship was obtained for seedling growth reductions and frequency of M_1 plants with somatic mutations. LD_{50} was found at a dose of approximately 1600 rads.

2. The high sensitivity of peanut seedlings and growing M_1 plants to fast neutrons after seed irradiation may have been due to the highly differentiated embryo at the time of treatment.

3. Radioresistance of peanut to the treatment, shown by low M_1 seedset reductions and low frequency of M_2 mutations, appears to be associated with its polyploid genome.

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Adoracion T. Arañez, Discussant

The study of Dr. Joventino D. Soriano on mutagenic response of peanut to fast neutrons may pave the way for the improvement of peanut varieties in the Philippines. According to Sigurbjornsson and Micke (1974), the technique of mutation breeding can be recommended to plant breeders as a practical tool with a reasonable guarantee of success. However, it appears that many plant breeders are unaware that the use of this technique has resulted in noteworthy plant breeding advances. They attributed this partly to the publication of works on induced mutations and mutation breeding in proceedings with distribution that is limited beyond their own circles. Mutation breeding may be done by direct multiplication of mutants or mutants may be used in cross breeding. The estimated value of induced mutant crop varieties now grown by farmers, shows that the net value of these crops outshines any cost that have gone into mutation breeding research. Around 145 mutant varieties are developed through the use of induced mutations as early as 1974. The mutagens used which have led to the development of superior varieties are X-rays, gamma rays, neutrons and chemical mutagens. Among the improved characters of crop varieties developed through induced mutations are higher yield, lodging resistance, disease resistance, early maturity, short stem, quality, winter hardiness, higher protein, shattering resistance, improved plant type and easier harvesting. Released varieties developed through induced mutations are cereals, legumes, fruit trees, ornamentals and other crops (Sigurbjornsson and Micke, 1974).

Researchers at the Philippine Atomic Energy Commission (PAEC) have produced through mutation breeding four new high yielding varieties of rice, four improved varieties of mungbean and a compact soybean mutant (PAEC Accomplishment Report, 1972-80).

A change in only one or few bases of the DNA may give rise to a mutant allele. A base substitution in the polynucleotide alters a codon. A different amino acid may be coded for by the new codon. The tertiary and quaternary configuration of a protein may be changed due to an alternation in the sequence of amino acids in the polypeptide. A change in the configuration of a protein may change its activity or property.

A study on human sickle-cell anemia showed that a change in one amino acid of a polypeptide chain is enough to produce an abnormal hemoglobin. The chemical abnormality of the sickle-cell hemoglobin resides in a change of a single amino acid. The 6th amino acid from the N-terminus of the β-peptide chain which is glutamic acid is replaced by valine. The triplet code for glutamic acid is GAA or GAG while that for valine is GUA or GUG (Goodenough and Levine, 1974). In either case, replacement of adenine by thymine will change the amino acid coded for by the triplet from glutamic acid to valine.

Gaul (1964) is of the opinion that plant breeding is controlled evolution and that in breeding, full use is made of two of the three main factors of evolution,

that is recombination and selection. He added that mutation, which is the third factor and a primary evolution factor can be used for breeding. The variations produced by mutagens are not essentially different from those caused by spontaneous mutation during evolution (Sigurbjornsson, 1970).

It is hoped that in the future, more researchers will go into the use of mutagens in producing genetic variations especially for plants that are usually propagated by asexual methods, those with long vegetative phase prior to sexual reproduction and in lower forms which are usually haploid and of which the only way to change the genetic material is through the use of agents that are capable of modifying the DNA.

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Prescillano M. Zamora, Discussant

In the work just presented by Dr. J.D. Soriano, which he undertook vis-a-vis a long-range program of mutation breeding, the application of fast neutrons (in varying doses) on dormant seeds of peanut has elicited the following responses: (1) seedling height was reduced with increasing radiation doses, (2) frequency of M_1 somatic mutations was increased with increasing radiation doses, (3) M_1 seedset was not reduced even in the dose above LD_{50} of 1600 rads, and (4) frequency of M_2 macro-mutations ranged from only 3.60 to 5.65/100 M_2 plants vis-a-vis the control plants.

In discussing the genetic basis of each of the foregoing radiation responses of the experimental material, Dr. Soriano stated that the high sensitivity of the M_1 seedlings and of the growing plants is probably due to the highly differentiated state of the embryo at the time of irradiation, while the radioresistance exhibited by the mature plants appears to be related to the polyploid genome of the experimental material.

With the foregoing remarks as term of reference, I now would like to focus my comments on the probable developmental anatomic basis for that mutagenic response relating to the increased frequency of M_1 somatic mutations with increasing radiation doses and the proposed explanation that said response is probably due to the highly differentiated state of the embryo at the time of the treatment.

Depending upon the variety, the fruit of peanut generally contains 2-4 seeds placed in tandem within a single locule. The seed of peanut (in the mature fruit) consists of a large embryo (ca 1.0 cm long x 0.7 cm wide) and a seed coat. Structurally, the peanut embryo is very similar to that of other species of legumes (Reed, 1924; Yarbrough, 1949; Smith, 1956). It consists of an axis, the hypocotyl-root axis bearing, at one end, the root apical meristem and, at the other, the two cotyledons and the apical meristem of the first shoot, the epicotyl. According to one report (Yarbrough, 1957), the peanut embryo has not only a leafy epicotyl but also two lateral shoot primordia arising at the cotyledonary axis in the mature seed.

Developmentally, the peanut embryo follows a pattern that resembles closely that of a dicotyledonous species (Smith, 1956). Although it develops a multicellular suspensor, the type of embryo which forms may be categorized as a common angiosperm type. Four or five days after pollination, the embryo consists of about 5 to 13 cells. Eight to ten days after pollination, it still has only about 5 to 15 cells. But it grows rapidly on about the 11th day after pollination, so that on about the 30th day after pollination, the embryo is, as described above, large with a short hypocotyl-root axis, bearing a well-organized apical meristem, a small epicotyl (plumule) also bearing a well-organized apical meristem and two large fleshy cotyledons.

The highly organized epicotylar apical meristem is interpretable according to the tunica-corpus concept of shoot apex (shoot apical meristem) organization. Accordingly, two tissue zones occur in the epicotylar apical meristem, namely, the tunica, consisting of two peripheral layers of cells, and the corpus, a mass of cells overarched by the tunica. The demarcation between these two zones results from the contrasting modes of cell division of the tunica and the corpus. The layers of the tunica show predominantly anticlinal divisions; that is, they undergo surface growth. The corpus cells divide in various planes, and the whole mass grows in volume. Each layer of the tunica arises from a small group of separate initials, and the corpus has its own group of initials located beneath those of the tunica. In other words, the number of tiers of initials is equal to the number of tunica layers plus one, the tier of corpus initials. The epidermis usually arises from the outermost tunica layer, while the underlying tissues may arise from the tunica or the corpus or both. Thus, the tunica-corpus concept is useful in relating the effects of irradiation treatments on the experimental material, in this case, the epicotylar apical meristem of the peanut embryo.

By the results of Dr. Soriano's treatments, somatic mutations consist mainly of leaf flecking and leaf sectoring in seedlings. Said forms of chimerism could result from the mutation of some of the initial cells at the apical meristem of the epicotyl that had been affected by the treatments applied, in this case fast neutrons. Said effects are then perpetuated with the subsequent division of the affected initial cells and these are expressed phenotypical as leaf flecking and leaf sectoring.

The foregoing is paralleled by induced cytochimeras produced with the use of colchicine (Satina *et al.*, 1940; Dermen, 1953, 1960; Clowes, 1961; others). Treatment of shoot and floral apices with colchicine, has resulted in the change of the number of chromosomes in individual cells as in the case of *Datura*, peach and cranberry from 2N to 4N and 8N. When cells occupying the position of initials in the shoot apex are thus affected the change becomes detectable and is perpetuated developmentally in more or less extended parts of the plant body that develop after the treatment, and the alterations had been traced directly to the initial cells in the apical meristems.

It is thus possible by these examples to support the view with high degree of confidence from the ontogenetic standpoint that the observed chimeric phenomenon brought about by fast neutron treatments is due to the activity of the affected initial cells in the apical meristem of the epicotyl of the peanut embryo.

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