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INDUCTION OF USEFUL MUTATIONS IN PEANUT (*ARACHIS HYPOGAEA* L.)[1]

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ABSTRACT

Pre- and post-irradiation treatments, close M_1 planting, continuous selection, and high infection pressure were employed. The mutations obtained were low growth habit, early flowering, long pod, large pod, big seed, white testa and resistance to the leafspot disease.

Introduction

The improvement of crops has undoubtedly been one of man's main concerns throughout the ages. Induced mutations have been particularly important when the needed natural germplasm has not been readily available. There are now about 500 cultivars which have reportedly been developed through induced mutations (Donini *et al.*, 1984) and many more will likely be released in the future as the search for new and improved methods of mutagenic treatment and mutant selection is strongly and consistently pursued.

A review of even most of the recent papers on induction of mutations through seed irradiation, however, did not show evidence of the use of experimental methods aimed at improving the chances of inducing and selecting the needed mutations. These methods have been derived from valid radiobiological concepts more commonly known as the oxygen effect (Caldecott and North, 1961; Nilan *et al.*, 1961; Nilan and Konzak, 1961), diplontic selection (Balkenia, 1972; Gaul, 1964), delayed segretation of induced mutants (Soriano, 1984), and application of high infection pressure for detection of disease resistance mutations (Micke, 1983). Many mutation workers may not have been informed at all of these useful developments in their work or are perhaps merely skeptical about the outcome of adding new inputs of time and resources in the use of experimental techniques such as pre- and post-irradiation treatments (Caldecott and North, 1961; Konzak *et al.*, 1961; Nilan *et al.*, 1961 Pathirana and Wijewickrama, 1983), close M_1 planting (Gaul,

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1964), continuing mutant selection from the M_1 to the M_3 generations and successive inoculations for screening disease resistance mutations.

This aspect of the work was conducted for the purpose of illustrating the main advantages to be gained from the use of these experimental techniques. The work consisted of four separate studies on peanut, as follows: (1) Effects of pre- and post-irradiation treatments on M_1 seedling height, seedset and frequency of M_2 seedling mutations; (2) Influence of wide and close planting on M_1 plant survival, seedset and frequency of M_2 seedling mutations; (3) Percentage of M_2 and M_3 lines segregating for some "off-type" M_1 characters or features; and (4) Comparison of the effectivity of low and high infection pressures for screening disease resistance mutations. Peanut is a good material for seed irradiation studies due to its unusually developed seed embryo which is well differentiated with a large plumule consisting of 10 leaves and 4 axillary buds (Emery, 1972). As the epicotyl does not produce any new parts during the first 3 weeks of growth, its seedling growth response to radiation at that age is more or less a faithful reflection of its true sensitivity to radiation treatment.

Materials and Methods

In the first study, dormant seeds of peanut, c.v. CES-3, were selected for uniform size and absence of deformities and were stored in a moist dessicator for seven days to maintain a uniform moisture content of about 14%. The seeds were then transferred to small plastic envelopes for treatment with varying doses of gamma radiation ranging from 15-60 Krads in a Gamma Cell Irradiation Facility at the Philippine Atomic Research Center. Untreated seeds from the same source were used as control. Within half an hour after radiation exposure, the seeds were rehydrated in water for two hours at a constant temperature of 30°C, rinsed briefly with tap water and sown on moist tissue paper in petri dish

For the data on seedling height, some of the seeds were planted on a "blotter sandwich" and grown in a plastic compartment for 14 days under conditions of high humidity, room temperature ranging from 28°C-30°C and constant flourescent illumination of about 80 foot-candles. The control (seed batch without pre- and post-irradiation treatments) were set aside in open plastic envelopes outside the glass dessicator for exposure to gamma radiation at the same time as the seed batch given the pre- and post-irradiation treatments. After radiation exposure, the seeds were sown directly on moist tissue paper in petri dish and later transplanted in field rows side by side with those given the pre- and post-irradiation treatments. At maturity, pods of M_1 plants picked at random were dehulled for seedset data. Later the M_2 seeds were sown in nursery rows for data on chlorophyll deficient mutations.

In the second study, the seeds were all given the same pre- and post-irradiation treatments and radiation doses as in the first study. One half of the seeds per dose were planted in field rows at distances of 30 cms. between rows and 10 cms. in the row for close planting. Just before harvest, plant survival was deter-

mined for the different doses and seedset data were obtained from pods of plants randomly marked in the field. The M_2 seeds were sown in nursery rows for determination of the type and frequency of M_2 seedling mutations.

In the third study, employing in the M_1 generation pre- and post-irradiation treatments, doses of 8-32 Krads gamma radiation and planting distances of 30 cms. between rows and 20 cms. in the row, 15 seeds from each M_1 plant were sown directly in field rows in a progeny performance test. Plants in each row comprised an M_2 family or line. Seeds of the original variety were used as control. The M_2 lines were scored for the segregation of one of the "off-type" M_1 characters or features such as low growth habit, early flowering, long pod, large pod and big seed. For the M_3 test, seeds of M_2 plants from lines that did not exhibit any of the mutant characters were similarly sown plant-to-row for the segregation test. M_3 plants from one M_2 plant comprised an M_3 line.

In the fourth study, seeds of M_1 plants given the same treatments, doses and planting distances as those in the third study, were sown directly in field rows in a plant-to-row plant and inoculated at the age of two weeks with spore suspensions of the leafspot disease caused by *Cercospora personata* (B. and C.) Ell. and Ev. and *C. arachidicola* Hori, previously cultured under sterile conditions in the laboratory in PDA-salt medium. The spore suspension was prepared by scraping off the black spore mass from the culture plate into a flask containing a solution of 10% glucose and 5% KH_2PO_4. The large spore masses were broken up into a fine suspension by passing the mixture in a homogenizer. Inoculation was done by scratching the leaf surface with a dissecting needle and placing one or two drops of the spore suspension on the wounded area preferably in the late afternoon when a night rain was not imminent. One and three inoculations for low and high infection pressures, respectively, were made at intervals of two weeks. The M_2 lines were scored for the appearance of the leafspot disease starting about two weeks from inoculation until maturity.

Results and Discussion

Pre- and Post-Irradiation Treatments

As shown in Table 1, the growth of peanut seedlings was markedly reduced at doses of 30-60 Krads when the seeds were not given the pre- and post-irradiation treatments. Marked seedling height reductions were obtained only at 60 Krads if the seeds were given such treatments. Moreover, the degree of reduction of seedling growth was much greater at doses of 45 and 60 Krads when the seeds were not given the pre- and post-irradiation treatments than when such treatments were employed. A similar situation was observed regarding M_1 seedset reductions in the two plant groups. At doses of 30-60 Krads gamma radiation, the reduction in seedset in plants that did not receive the pre- and post-irradiation treatments was much greater than in plants receiving the treatments. Based on the seedling height and seedset data, therefore, exposure of peanut seeds to gamma radiation without pre-

Table 1. M_1 seedling height and seedset and M_2 mutation frequency in peanut with or without pre- and post-irradiation treatments

Gamma radiation dose	*Without Pre- and Post-Irradiation Treatment*			*With Pre- and Post-Irradiation Treatment*		
	Mean seedling height (% control)	*Mean seedset (% control)*	*Mutants per 1000 plants*	*Mean seedling height (% control)*	*Mean seedset (% control)*	*Mutants per 1000 plants*
0 (control)	100.00	100.00	0	100.00	100.00	0
15 Krads	100.00	88.36	7.85	100.00	100.00	16.28
30 Krads	67.84	64.25	16.82	86.37	87.29	29.62
45 Krads	48.73	51.48	11.33	73.64	78.19	72.46
60 Krads	31.56	18.62	8.74	61.87	66.84	68.75

and post-irradiation treatments caused markedly greater radiation injury at doses of 30-60 Krads than when given such treatments.

As to M_2 mutation frequency, a much higher mutation rate was found in plants that received the pre- and post-irradiation treatments (Table 1). Without such treatments, the number and frequency of seedling mutations was only about one-eighth to one-half of the seedling mutations in doses that received said treatments. The importance of this data to the mutation breeder is that the seedling mutation frequency has been found to be proportional to the frequency of useful mutations (Donini *et al.*, 1984; Gaul, 1964; Micke, 1983). It is very possible that the low mutation frequency in plants that did not receive the pre- and post-irradiation treatments is due to the high degree of radiation injury incurred by such plants particularly from peroxides formed from the combination of oxygen with the induced free radicals (Nilan *et al.*, 1961; Nilan and Konzak, 1961). Mutated sectors in the seed embryo and in the seedlings would fail to reach the flowering stage if the plant bearing them is heavily damaged or injured. Those that reach the flowering stage have a very low probability for transmission to the progeny due to the high lethality in seed formation. The use of chlorophyll deficiency seedling mutations as an index of the frequency of useful mutations in advanced generations has been indicated by previous studies (Caldecott and North, 1961; Gaul, 1964; Konzak *et al.*, 1961 Nilan *et al.*, 1961; Nilan and Konzak, 1961; Soriano, 1959). In peanut, the main types of seedling mutations obtained in the order of their relative frequencies were albina, xantha, viridis, maculata and albo-viridis scored according to the report of Gaul (1964).

Wide and Close M_1 Planting

The effects of wide and close distance planting on M_1 plant survival, seedset and M_2 mutation frequency, are shown in Table 2. No difference in plant survival was found between plants grown at wide intervals of 30 cms. and those at close intervals of 10 cms. Likewise, no difference in M_1 seedset was observed in the two treatments. This may be due to the low growth habit of the peanut plant which reduces the effects of overcrowding and competition known to occur in taller plant species. The stalks or branches of the plant spread out more or less radially and as the plant occupies an area of only about 15-20 cms. in diameter, only the tips of the stalks of neighboring plants overlap. The primary stalks bearing the underground fruiting nodes are generally erect and are relatively free from shading by neighboring plants.

Intrasomatic or diplontic selection is based on the idea that the plant originating from an irradiated seed is composed of normal and mutated sectors. Sectoral (mericlinal) chimerism, however, is not stable and is lost usually during the ontogeny of the plant (Balkenia, 1972; Gaul, 1964) through a process of competition in which mutated cells are at a disadvantage in favor of normal cells or tissues. Natural selection will eventually eliminate the mutated cells in the meristem. By growing the M_1 plants close together, a condition of overcrowding

Table 2. M_1 plant survival and seedset and M_2 mutation frequency in peanut after wide and close distance planting

Gamma radiation dose	*Wide distance planting*			*Close distance planting*		
	M_1 plant survival (% control)	*M_1 seedset (% control)*	*Mutants per 1000 plants*	*M_1 Plant survival (% control)*	*M_1 seedset (% control)*	*Mutants per 1000 plants*
0 (control)	100.00	100.00	0	100.00	100.00	0
15 Krads	99.43	99.56	6.78	97.58	96.37	10.72
30 Krads	83.72	86.67	21.82	81.82	84.28	26.41
45 Krads	65.87	75.44	16.35	64.81	72.66	61.63
60 Krads	59.38	60.16	11.43	56.47	59.35	68.56

and partial shading would prevail which is not conducive to the growth of normal tissues. This situation more or less gives the mutated sectors an equal advantage over the normal tissues.

To determine the proportion of germ track cells bearing a mutation in the generative inflorescence tissue, Gaul (1964), proposed the formula $u = m/f$, where u is the percentage of germ track cells bearing a mutation; m, the M_2 mutation frequency; and f, the average segregation ratio of chlorophyll mutations which is approximately 20%. Thus if the mutation frequency is 6% after seed-irradiation, about 3% of the germ track cells bear a mutation. Determination of this value gives a concept of quantifying the number of mutated cells due to different levels of radiation doses; otherwise, the formula is of little significance considering the many conditions that influence the fate of mutated cells in the generative tissue of the plant.

As to M_2 mutation frequency, close planting gave a much higher rate as compared to those growth far apart by a factor of 5 to 6 at the higher dose levels. Moreover, the mutation rate in plants grown far apart tended to decline at the dose of 45 Krads while in those grown close together, the mutation frequency continued to increase. As radiation doses were the same, this situation could not be due to the number of mutated cells in the seed embryo *per se* but invariably to intrasomatic selection in favor of the mutated meristem cells.

Delayed Segregation of Induced Mutant Characters

The idea of delayed segregation of induced mutant characters is based on the observation that many such characters while not appearing in the M_1 and M_2 generations are exhibited in latter generations. Table 3 shows the frequency of five induced "off-type" characters or features observed in 765 plants out of a total of 14,731 M_1 plants. Of these, "off-types", low growth habit appeared with the highest frequency of about 3.27% followed by long pod, 1.72%; big seed, 0.50%; large pod, 0.42% and early flowering, 0.13%. The "off-types" are described briefly as follows:

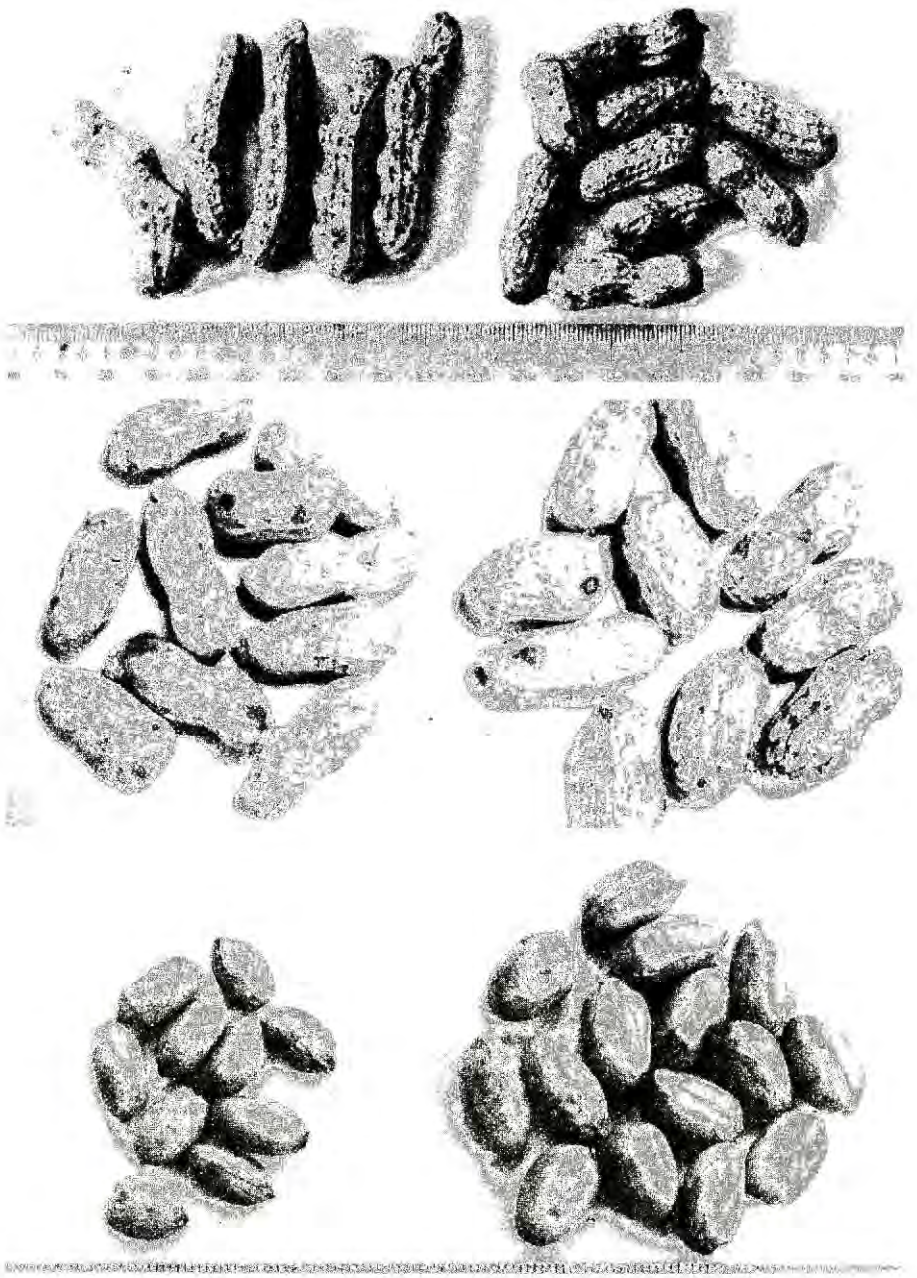
Low growth habit – The plants are short, growing only up to about one-half of the control; have much smaller stalks and smaller leaves.

Early flowering – The plant opens its first flower in not more than 42 days after planting, 10-15 days earlier than the mother variety.

Long pod – The pod consists of 4-5 locules compared to 1-3 locules of the mother variety and are visibly two to three times longer than average-sized pods of the control (Fig. 1).

Large pod – This big-sized pod has a diameter ranging from 1.6-1.8 cms. at its biggest girth compared to 1.1-1.3 cms. of the control (Fig. 2).

Big seed – Seed weight ranges from 64-83 grams per 100 seeds compared to only about 28-40 grams per 100 seeds of the mother variety. They are distinctly much bigger than the average-sized seeds of the control (Fig. 3).



Induced mutations in peanut: Fig. 1, Long pod: Fig. 2., Large pod; Fig. 3., Big seeds.

Table 3. Frequency of five "off-type" plant features in M_1 peanut after gamma seed-irradiation

Radiation dose	*Total M_1 plants*	*Low Growth*		*Early Flowering*		*Long Pod*		*Large Pod*		*Big Seed*	
		No.	*%*	*No.*	*%*	*No.*	*%*	*No.*	*%*	*No.*	*%*
0 (control)	2,603	0		0		0		0		0	
8 Krads	4,428	0		3	.07	0		0		5	.11
16 Krads	3,195	38	1.19	11	.34	26	.81	8	.25	3	.09
24 Krads	4,264	147	3.45	17	.40	54	1.26	12	.28	28	.66
32 Krads	2,844	211	7.42	6	.21	129	4.54	26	.91	41	1.44

These "off-types" were chosen for the segregation test due to their being easily detected. Furthermore, these M_1 plant features have the likelihood of being genetic characters. In peas, induced early flowering and seed size were found to be genetically controlled while in groundnut or peanut, large pod and early maturity were reported to be genetic characters (Patil *et al.*, 1982; Vassileva *et al.*, 1983). Also in groundnut, pink seedcoat and small pod have been confirmed to be induced genetic characters (Pathirana and Wijewickrama, 1983).

The percentage of segregating lines in the M_2 and M_3 generations are shown in Table 4. The similarity in total number of lines in both generations is due to the use of the same land area during the three growing seasons of the study. The number of segregating lines in the M_3 generation was more than twice that of the M_2 generation, *i.e.* 3.60% and 6.40%, respectively. The frequency of segregating lines in one generation varied widely according to the type of mutation. In the case of early flowering, a physiological character known to be genetically controlled, none of the 127 M_2 lines gave the mutation but this character appeared in the M_3 generation.

The peculiarity of this kind of work is the disregard of all the other mutation types except those under study. Large pod appeared the most frequently segregating type in both generations. Two useful mutations that appeared only in the M_2 generation are dark-green plant and white testa, in addition to a wide range of lethal, semi-lethal and unproductive plant types commonly encountered in a population with a mutagen-treated background.

The delay in the segregation of some induced mutations may be due to the highly heterozygous and heterogeneous nature of the irradiated plant and its progeny. Although most induced mutations are genetically monogenic, many have been found to be controlled by duplicate genes (Dubov, 1984; Patil *et al.*, 1982; Soriano, 1984) while others are multi-allelic (Donini, 1984; Flor, 1955; Gregory, 1956; Palmer *et al.*, 1978), polygenic (Donini, 1984) and epistatic (Genova, 1984) as well. In the groundnut, most of the induced mutations were found to be single traits (Pathirana and Wijewickrama, 1983). As an induced mutation of the monogenic type in the heterozygous state, two or more generations are needed for it to segregate depending on the genotypes of the generative cells producing the gametes involved in fertilization. A pistillate cell homozygous for the wild-type gene and a staminate cell heterozygous for the mutant recessive gene will necessarily delay the appearance of the mutation. Mutations that require additive action of many genes as well as those governed by duplicate or epistatic factors will likewise delay the segregation of the mutant character.

Some morphological features of the plant could undoubtedly contribute to the delay in appearance of an induced mutation. The occurrence of induced semi-lethal structures could reduce the chances of a mutation appearing in the next generation. A "cleansing on adjustment process" is indeed thought to occur in the mutagen-treated individual and its immediate progeny leading to the elimination of structures of negative selective value. Through cell, tissue and embryo

Table 4. Percentage of M_2 and M_3 lines of peanut segregating for induced mutations

Mutant Character	*M_2 Generation*			*M_3 Generation*		
	Total lines	*No. of segregating lines*	*% segregating lines*	*Total lines*	*No. of segregating lines*	*% segregating lines*
Low growth	174	3	1.72	175	8	4.57
Early flowering	127	0	-	128	3	2.34
Long pod	165	7	4.24	209	14	6.70
Large pod	206	12	5.83	216	21	9.72
Big seed	218	10	4.59	164	11	6.70

culture, man has successfully rescued lethal and inviable genotypes from extinction and many of them have been shown to possess distinct properties of some value to man (Micke, 1983).

In wheat, resistance to the leaf rust disease obtained from micro-mutant lines previously selected for other characters have been referred to as being a secondary mutations (Borojevic, 1975) as they did not appear in the progeny of the treated generation. Recurrent selection is a well-recognized practice in plant breeding and its efficiency in accelerating the improvement of crops has been well proven (Hristova and Hristov, 1984). In fact in the spring wheat (McNeal *et al.*, 1978), the second selection cycle progeny gave higher protein percentages than the first cycle progeny. Especially for detection of mutants with improved quantitative characters where mutagenic treatment might more often cause genetic changes with small effects, recurrent selection has been recommended to cope with the delayed segregation of such mutations (Barton *et al.*, 1980; Gaul *et al.*, 1976; Gregory 1956; Hana, 1982).

High Infection Pressure for Screening Disease Resistant Mutations

As shown in Table 5, more disease resistance mutations were obtained upon using a high infection pressure of three successive inoculations than a low infection pressure of only one inoculation. Out of a total of 6,555 M_2 plants subjected to only one inoculation, only about 0.52% exhibited the low degree of resistance as shown by the absence of leafspots on the three uppermost leaves. With three successive inoculations of the causal spores, however, about 1.29% of the plants exhibited resistance to the disease. This is almost two and a half times more than what was uncovered by low infection pressure.

Three levels of resistance to the leafspot disease have been assumed based on field observations and were tentatively given the following symbols:

r0 = highly susceptible plant. The entire plant exhibits symptoms of the disease including a total defoliation and stalk decay at maturity.

r1 = low resistance. The three uppermost leaves do not show any disease symptoms but defoliation is prevalent at maturity.

r2 = intermediate resistance. The five uppermost leaves are free of any disease symptoms. Defoliation is very light with only the older lower leaves falling off at maturity.

r3 = high resistance. The entire plant does not exhibit any of the above symptoms of the disease.

The effectivity of high over low infection pressure for screening disease resistance mutations is shown not only by the number of plants exhibiting various levels of reaction to infection but also by the quality of resistance obtained. Low infection pressure appears to have been capable of uncovering only the low resistance mutation but not the high resistance mutation. It is possible that a low infection level fails to express the full potential of the genotype in a similar way

Table 5. Frequency and types of disease resistance mutations in M_2 peanut obtained after one and three inoculation of leafspot fungus spores

Planting season	*One inoculation*			*Three inoculation*		
	Total M_2 plants	*% without symptoms*	*Type of resistance*	*Total M_2 plants*	*% without symptoms*	*Type of resistance*
May, 1984 – Aug., 1984	1,827	0.60	All r^1	1,374	1.82	All r^2
Sept., 1984 – Dec., 1984	2,546	0.82	13 r^1 1 r^2	2,709	1.25	All r^2
Feb., 1985 – May, 1985	2,182	0.41	All r^1	2,453	1.06	17 r^2 9 r^3

Legend:

r^1 = low resistance
r^2 = intermediate resistance
r^3 = complete resistance

that only an intermediate-sized maize ear (Brinkerhoff, *et al.*, 1978), also a quantitative character, develops if the conditions for growth are limited inspite of the superior genotype of the plant.

Low infection pressure, however, has some distinct advantages (Chopra, 1980; Donini *et al.*, 1984) of no small value to mutation breeders. Heavy inoculation densities may create a disease epidemic that could mask identification of plants with low and partial resistance to the disease. The method to be employed may seem to depend on the nature of the disease itself. In cotton, an induced mutation for resistance to bacterial blight showed only an intermediate level of reaction to the disease and was subsequently found to be due to a single dominant gene (Brinkerhoff *et al.*, 1978). In peanut where the primary criterion for scoring reaction to the leafspot disease is leaf defoliation, (Conger and Gregory, 1980), only the genotype for total resistance would be adequate to minimize yield loss due to infection. As this appears to be a polygenic character (Cooper and Gregory, 1980; Gregory, 1956), the need for continuous or recurrent selection for several generations is indicated.

In choosing between the use of low or high infection pressure for screening disease resistance mutations, peanut should be considered an exception because low or partial levels of disease resistance to the leafspot disease cause heavy and moderate defoliation, respectively, both resulting in yield reductions. Inasmuch as high yield is the primary goal of mutation breeding in this crop species, a high infection pressure seems to be the more appropriate means of attaining that objective.

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Summary and Conclusions

In efficient induction of useful mutations in peanut through seed irradiation, the following experimental techniques were found useful for increasing mutation frequency and screening useful mutant types:

1. Employment of pre- and post-irradiation treatments to minimize radiation injury in M_1 plants and increase mutation frequency in the M_2 generation;
2. Close M_1 planting to shift diplontic selection in favor of mutated cells and sectors in the M_1 meristem leading to an increase in M_2 mutation frequency;
3. Continuous or recurrent selection of mutant characters from the M_1 to the M_3 generations through a close follow-up of M_1 "off-types" or selections in a progeny performance test with a plant-to-row planting plan; and

4. Application of a high infection pressure for screening the high level resistance mutations where heavy disease infestation causes large scale defoliation and yield loss.

The advantages gained from the use of the above methods and their fundamental bases are briefly discussed.

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