MUTATIONAL IMPROVEMENT OF PEANUT[1]

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ABSTRACT - A study was initiated to improve the peanut variety Tatu (*Arachis hypogaea* L., ssp. *fastigiata* Waldron, var. *fastigiata*), through induction of mutations for increased pod number per plant and better pod filling, using a chemical mutagen sodium azide (NaN_3). The treatment with sodium azide at a concentration of 3mM was found to be the most efficient as well as effective for induction of desirable mutants. Among the ten productive mutants selected with varying degrees of improvement over the parent for the characters mentioned above, three of them Dwarf, Sd-HP and V_3 consistently maintained their superiority in yield at significant levels over a period of three seasons including conditions of low rainfall. The mutants Dwarf and Sd-HP demonstrated improvement in pod number per plant, seed number per pod, shelling percentage and harvest index in the M_4 generation and superior yield levels. Use of these mutants in peanut breeding is discussed.

Index terms: *Arachis hypogaea* L., mutation, peanut breeding.

MELHORAMENTO MUTACIONAL DO AMENDOIM

RESUMO - Foi iniciado um estudo visando melhorar a variedade Tatu de amendoim (*Arachis hypogae* L., ssp. *fastigiata* Waldron, var. *fastigiata*) através da indução de mutações para maior número e melhor enchimento de vagens, utilizando o mutagênico químico azida de sódio (NaN_3). O tratamento na concentração de 3mM foi mais eficiente e efetivo em indução de mutantes desejáveis. Dos dez mutantes com vários graus de melhoramento para as características estudadas, como Anã, Sd-HP e V_3, mantiveram, coerentemente, a superioridade em produção durante três estações, inclusive nas condições de pouca chuva. Na geração M_4, os mutantes Anã e Sd-HP mostraram ganhos em número de vagens por planta, número de sementes por vagem, proporção de sementes por vagem e índice de colheita, além de altos níveis da produção. O presente trabalho trata sobre o aproveitamento destes mutantes em melhoramento genético do amendoim.

Termos para indexação: *Arachis hypogaea* L., mutação, melhoramento genético.

INTRODUCTION

Despite cultivation of peanut (*Arachis hypogaea* L.) in eleven states of Brazil, production of this crop in the country has been declining since 1972 (Pompeu 1980). A shift in the governmental priorities to encourage cultivation of more remunerative crops had resulted in decline in the area under peanut in the state of São Paulo, a principal producer of the crop. Another reason for this situation could perhaps be the lack of appropriate peanut varieties suited to diverse agroclimatic regions of the country.

The predominant peanut variety Tatu, for example, was found to be inferior in yield to a North American variety Florispan under semi-arid conditions of Ceará (Pereira 1976).

Alteration of specific attributes of crop varieties through induced mutagenesis is a well known method of plant breeding adapted in various crops including peanut (Brock 1980, Nilan et al. 1977 and Prasad et al. 1984). A study was therefore initiated to improve the variety Tatu through induction of mutations for larger number of pods and higher number of seeds per pod employing a chemical mutagen sodium azide which was found effective in barley (Nilan et al. 1976) and rice (Awan et al. 1980).

MATERIAL AND METHODS

Three hundred well developed dry seeds of the peanut variety (*Arachis hypogaea* L., ssp. *fastigiata* Waldron, var. *fastigiata*) per treatment were presoaked in water for 23 hours and treated for 2 hours and 30 minutes at room temperature, with a solution of sodium azide (NaN_3) of

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concentrations of 2mM, 3mM and 4mM, prepared by dissolving the chemical of corresponding weight in 0.1M Citric acid - Sodium phosphate buffer solution adjusted to pH3. After the treatment, the seeds were thoroughly washed in running tap water for 20 minutes and immediately sown together with the appropriate control, in a well prepared field with a spacing of 60 cm between rows and 30 cm between the seeds within a row. The same spacing was adopted throughout the study.

The M_1 and M_2 generations were raised in the fields of the Department of Plant Science of the Centre of Agricultural Sciences of the Federal University of Ceará in Fortaleza. The subsequent M_3 and M_4 generations were grown in the fields of the Coastal Research Unit of EPACE at Pacajus, Ceará. The M_1 and M_2 generations were handled in the usual way (Prasad 1972). In the M_1 generation, seed sterility was estimated as percent of reduction of seed number in relation to control.

In the M_2 generation, each plant was thoroughly examined for pod weight and pod number and only those plants with a pod weight of over 33 g and an effective pod number of over 40 were selected. The selected mutants were identified by abbreviations as indicated in Tables 2 and 3 based on the extent of their resemblance to the parent at the time of selection. The mutants S_1, S_2, S_3 and S_4 were closer to the parent in canopy characters, but differred in pod number. HP_1 -V exhibited a reduction number of branches and a higher pod number, while HP_2 showed a higher number of pods and a slight variation of plant canopy. The mutants Sd-HP, Dwarf, V_3 and V_4 exhibited minor variations in plant canopy together with seed and pod characters, height and number of branches respectively. The progenies of the selected M_2 plants were evaluated in the M_3 and M_4 generations along with the parent variety Tatu, in a randomised block design with three and four replications and with net plot sizes 1 row and three rows of 3 m length each, respectively.

While mutagenic effectiveness is the ratio between the percentage of M_2 families segregating for mutations and the product of time of mutagenic treatment and concentration of the mutagen, mutagenic efficiency is the proportion of M_2 families segregating in relation to the percentage of sterility induced by the mutagen. In the present investigation, modified formulae of Konzak et al. (1965) as adopted by Prasad (1972) were used to calculate the values of mutagenic effectiveness and efficiency of the three concentrations of sodium azide with regard to the incidence of the mutants for higher pod yield alone (Table 1).

The data on various characters reported in the study except yield, were collected from five randomly selected plants of each plot, while yield of all plants in each plot was averaged out to pod yield per plant. Harvest Index was calculated as percentage of seed weight to the total dry weight of plant at harvest. Shelling percentage was estimated as the proportion of seed weight to the pod weight.

The crop received a total of four irrigations including pre-sowing irrigation, in each of M_1, M_2 and M_3 generations, but was grown under rainfed (rain fall: 313 mm) conditions in the M_4 generation. In the M_2 and M_3 generations, the third and the fourth irrigations were given at forty (40) and seventy (70) days respectively after sowing, to simultate moisture stress at pod development stage of the crop. In the M_4 generation, bulk of the rainfall was received before the pod development and filling stages. Therefore in the M_2, M_3 and M_4 generations, the crop was subjected to moisture stress at pod development stage, which was reflected in the lower number of pods per plant, as well as poor seed filling in the variety Tatu.

Throughout the study the crop was fertilized at a rate of 20 kg N, 60 kg P_2O_5 and 40 kg K_2O per hectare at sowing and at a rate of 10 kg N per hectare at flowering. Appropriate cultural and plant protection operations were carried out when ever necessary.

TABLE 1. Effects of mutagenic treatments in M_1 and M_2 generations.

Treatment	M_1 generation		M_2 generation			Mutagenic effectiveness * Me/tc**	Mutagenic efficiency * Me/S***
	Germination %	Seed sterility	Number of plants	Number of productive mutants selected	Families showing mutants %		
1. Tatu-Control	100.0	0.0	3,007	-	-	-	-
2. Tatu-NaN3-2mM	85.0	23.1	2,316	2	0.8	0.16	0.35
3. Tatu-NaN3-3mM	84.4	28.7	2,143	4	1.6	0.21	0.56
4. Tatu NaN3-4mM	75.0	35.9	1,925	4	1.8	0.18	0.50

* Me = percentage of M_2 families segregating for the mutants

** tc = duration of mutagenic treatment (t) x concentration of mutagen (c)

*** S = percentage seed sterility in M_1 generation

TABLE 2. Data on pod yield and some yield components of the selected M_2 mutants and the parent.

Mutant/parent	Mutagenic treatment in M_1 generation	Pod weight per plant (g)	Pod number per plant	Mean seed number per pod
1. S1	NaN3-3mM	56.0	51	1.6
2. HP1-V	NaN3-3mM	68.0	62	1.5
3. HP-2	NaN3-4mM	58.0	52	2.0
4. S2	NaN3-3mM	80.0	77	1.8
5. S3	NaN3-2mM	73.5	70	2.0
6. S4	NaN3-3mM	82.5	81	2.5
7. Sd-HP	NaN3-4mM	78.0	69	2.2
8. Dwarf	NaN3-3mM	33.0	40	2.1
9. V3	NaN3-4mM	37.0	42	2.2
10. V4	NaN3-4mM	53.0	51	2.5
11. Tatu (Parent)	-	31.0	33	1.1

RESULTS AND DISCUSSION

Germination and seed sterility in M_1 generation:

From the Table 1 it can be observed that following the treatment of sodium azide, seed germination reduced progressively with the increase in concentration of the mutagen. While the lowest concentration 2mM brought about 15% reduction in germination, it was reduced by 25% in the highest concentration of 4mM. The concentration of 3mM however was less drastic resulting in only 15.6% reduction in germination.

More or less a same trend was observed with regard to the incidence of seed sterility in the M_1 generation following the treatment of sodium azide of varying concentrations (Table 1). While all the three concentrations conspicuously reduced seed fertility, the highest concentration was the most drastic, showing 35.9% sterility. The lowest concentration 2mM, resulted in 23.1% seed sterility. The reduction in fertility in the case of 3mM concentration was however, not too drastic, although higher than that of 2mM.

Various physical and chemical mutagens have been known to affect germinability of seed and fertility with increase in concentrations. Results reported in the study are in general in agreement with those of Niknejad (1976) in barley, Levy & Ashri (1973) in peanut and Awan et al. (1980) in rice with sodium azide, although the concentrations used in this study were higher, resulting in more drastic reductions in germination. Despite a similar trend, the degrees of sterility observed in the present investigation were, however, much lower than those reported by Awan et al. (1980). Since sodium azide does not induce chromosomal aberrations (Niknejad 1976 and Walther 1976), the sterility observed could be attributed to gene mutations expressed as gametic or zygotic lethals (Nilan et al. 1976).

Mutation frequency in M_2 and mutagenic effectiveness and efficiency

Considering the population size and incidence of mutants for higher pod number and pod weight in the M_2 generation (Table 1), it could be observed that despite a reduction in the M_2 population sizes, at higher concentrations, it was possible to select higher number of mutant plants, than in the case of 2mM concentration. Similarly a relatively higher number of M_2 families segregated for the desirable mutants selected in the case of two higher concentrations. Awan et al. (1980) based on their studies in rice, reported very high frequences of chlorophyll mutations (14%) and various viable mutations (1.9 - 4.6%) induced by sodium azide. In the present investigation, however, chlorophyll and viable mutants other than the mutants for higher pod yield, were not screened for, in view of the specific aims and objectives of the study. A comparison of mutation frequencies of rice with those of peanut, despite use of the same mutagen, would not be meaningful in view of their divergent genetic constitutions, including differences in the levels of ploidy. Nevertheless, considering mutation frequencies reported in peanut using other physical and chemical mutagens (Prasad et al. 1984), the frequencies of productive mutants induced by sodium azide in the present study were slightly higher than the frequencies of similar mutations induced by the chemical mutagens such as ethyl methane sulfonate and nitroso methyl urea.

Mutagenic effectiveness and efficiency are the criteria adopted to determine the usefulness of a mutagenic treatment for plant breeding programs (Konzak et al. 1965). A mutagenic treatment should be the most efficient as well as effective in order to find use in plant breeding programs. It can be observed from the data that the treatment of sodium azide at the concentration of 3mM was effective and efficient, in view of the moderate degree of sterility and a high proportion of M_2 families segregating for the productive mutants that occurred in this treatment, closely followed by that of 4mM, the efficiency of which could have been higher, but for the highest degree of seed sterility.

Agronomic value of the mutants:

A perusal of the data presented in Table 2 indicates that the selection in the M_2 generation was based on number and weight of effective pods, as it was expected to be accompanied by an increase in seed number also (Basu & Ashokraj 1969, Coffelt & Hammons 1974). A modest enchancement of pod weight and number, but a conspicuous increase in seed number over the parent were observed in the case of the two mutant plants viz., Dwarf and V_3, while the others such as S_4, S_2, Sd-HP, S_3, HP.$_2$, S_2 and V_4 exhibited striking improvement in all the three characters. The remaining two mutants HP_1 -V and S_1 revealed perceptible increases in pod number and weight, but a moderate enhancement of seed number.

The data on pod yield presented in Table 3 indicate that all the mutants except HP_1 -V maintained their striking superiority over the parent in the M_3 generation also. The range of yields of the nine superior mutants in the M_3 generation varied from 41.6 g to 63.7 g per plant as against the parental value of 16.4 g per plant. In the M_4 generation, the yield patterns (Table 3) of the mutants and the parent were different when the crop was grown under unirrigated conditions. Although all the ten mutants gave higher yields than the parent which yielded 24.7 g per plant, only four mutants such as Dwarf (64.0 g), Sd-HP (51.4 g), V_3 (46.7 g) and HP_1 -V (38.0 g) significantly outyielded the parent. It is interesting to note that the three mutants such as Dwarf, Sd-HP and V_3 maintained consistently high levels of performance in yield in both M_3 and M_4 generations, when the crop was grown under different conditions of management. Yields of high order exhibited by these three mutants under low rainfall (313 mm) conditions characterised by drought at pod development stage, indicate their tolerance to the conditions of moisture stress. Earlier studies carried out on green gram (*Vigna radiata*) (Prasad 1976) brought out the feasibility of chemical mutagenesis in developing new plant types possessing drought tolerance together with high yield potential without drastically altering an otherwise well adapted plant genetic constitution. The data on mean yields of the two seasons presented in Table 3, further reveal the superiority of Sd-HP and Dwarf mutants, notwithstanding high mean yields of all the rest of the mutants as compared to that of the parent. The mutant HP_1 -V exhibited a variable performance characterised by and inferior level of yield in the M_3 generation and a significantly superior yield, though not of a very high order, in the M_4 generation. The variable performance of the mutants reveals their diverse yield potentials brought about through different mutations without drastically altering the genetic background of the peanut variety Tatu. Such mutant types could be of use in genetic and plant breeding investigations (Emery et al. 1965 and Nilan et al. 1977).

Considering the yield components (Table 3), all the mutants in the M_4 generation except HP_1 - V maintained a significantly higher number of pods per plant over their parent. In spite of a higher number of pods per plant in the case of HP_2 and S_2, they exhibited a much lower proportion of effective pods per plant (i.e., pods containing seeds). On the other hand, the mutants Dwarf and

TABLE 3. Data on performance of the induced mutants and parent.

Mutants/ parent	Treatment in M_1 generation	Data of M_4 generation on								Pod yield per plant (g)		
		Days to flowering	Days to maturity	Pod number per plant		Seed number per pod	Weight of 100 seeds g	Shelling percent	Havervest index (%)	M_3 generation	M_4 generation	Mean
				Total	Effective							
1. S1	NaN3-2mM	26	97	45.0	32.2	1.25	50.3	62.1	27.7	49.3	32.6	41.0
2. HP1-V	NaN3-3mM	30	98	21.0	20.3	1.20	45.0	33.5	13.6	16.0	38.0	27.0
3. HP2	NaN3-4mM	30	98	68.3	36.1	1.50	44.6	76.7	33.0	63.7	31.4	47.6
4. S2	NaN2-3mM	28	97	60.4	32.4	1.40	44.5	63.6	24.2	60.7	31.7	46.2
5. S3	NaN3-2mM	30	98	43.7	27.8	1.50	44.7	58.2	17.9	46.0	32.0	39.0
6. S4	NaN3-3mM	27	97	48.3	27.1	1.50	42.0	57.1	23.1	48.3	29.8	39.1
7. Sd-HP	NaN3-4mM	26	95	55.6	44.8	2.00	41.5	72.4	33.3	58.0	51.4	54.7
8. Dwarf	NaN3-3mM	26	95	53.3	53.1	2.00	40.8	77.5	35.7	60.0	64.0	62.0
9. V3	NaN3-4mM	25	96	51.0	50.5	1.33	40.5	62.2	24.6	41.6	46.7	44.2
10. V4	NaN3-4mM	26	96	49.0	32.3	1.80	41.5	74.8	26.5	62.3	30.6	46.5
11. Tatu (parent)	-	28	103	20.1	20.0	1.17	42.7	40.7	10.3	16.4	24.7	20.6
SEm ±				4.9	3.7	0.14	0.8			2.8	3.2	
CDat 5%				15.2	11.8	0.46	2.3			8.5	9.3	

V_3 showed the highest proportion of effective pods per plant. However, the mutants Dwarf, V_3, Sd-HP, HP_2, S_2, V_4 and S_1 showed significantly higher number of effective pods per plant than the parent Tatu. Though the mutants showed increased seed number per pod in the M_4 generation, only Sd-HP, Dwarf and V_4 were significantly superior to the parent in this character. Regarding the 100 - seed weight, only two mutants S_1 and HP_1-V with 50.3 g and 45.0 g respectively were significantly superior to the parent with 42.7 g per 100 seeds. The values of 100-seed weight in respect of HP_2 (44.6 g), S_2 (44.5 g) and S_3 (44.7 g), nevertheless, were close to statistical significance. All the mutants, except HP_1 -V, exhibited a higher shelling percentage (57.1 to 77.5) over the parent, with a value of 40.7 (Table 3). All of the mutants were observed to be comparable to the parent in maturity.

Additionally, it can be observed from Table 3 that all the mutants, except HP_1 -V and S_3, exhibited an improvement in their harvest indices (24.2% to 35.7%) over the parent, with a value of 10.3%. The values of harvest indices were the highest in the cases of Dwarf (35.7%) and Sd-HP (33.3%) mutants. Such a favourable partitioning of a high proportion of the plant dry matter towards economic yield of plant through gene mutations could be an effective means of developing peanut varieties with higher degrees of productivity (Swaminathan & Jain 1973).

The doubling of peanut yields over the past 40 years in the USA has been accomplished through the development of varieties which partition more of the assimilate to the fruit, but not through corresponding increases in crop growth rate (Ducan et al. 1978) leading to the increase in the number of fruit sites (Wynne & Gregory 1981). Therefore it would be necessary to enrich the peanut germplasm with new genes for productivity. In this context, new peanut mutants with higher yield potentials of diverse nature might help plant breeders gain greater insight into possibile structural alterations in peanut plant for increasing fruit production as suggested by Wynne & Gregory (1981).

The peanut mutants reported in the study may be tested further in different regions of semiarid tract of Northeastern Brazil, in view of their encouraging performance under unirrigated conditions characteristics by soil moisture stress. It would also be interesting to examine the quantity and quality of oil of these mutants, to understand the effect of these gene mutations on the traits pertaining to the content and composition of peanut oil.

CONCLUSIONS

1. Among the three different concentrations 2mM, 3mM and 4mM of the chemical mutagen sodium azide employed in peanut variety Tatu, the treatments with 3mM and 4mM concentrations were found to be more efficient and effective, in that order. The efficiency of the concentration 4mM was a bit lower than that of 3mM due to the highest degree of seed sterility observed in the M_1 generation of this treatment.

2. In both M_3 and M_4 generations, the three mutants viz., Dwarf, Sd-HP and V_3 consistently maintained their significant superiority in yield over the parent.

3. The two productive mutants Dwarf and Sd-HP also exhibited significant and striking improvement in pod number per plant, seed number per pod, shelling percentage and harvest index in the M_4 generation as compared to the parent.

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