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Induced mutations in cowpea, *Vigna unguiculata* (Leguminosae)

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Abstract: Two cowpea *(Vigna unguiculata L. Walp)* varieties, IT84E-124 and Vita 7 of the International Institute of Tropical Agriculture, Ibadan, were exposed to varying doses of chemical and physical mutagens. Optimum doses of 10mM EMS for 6hr and 0.1mM and 1.0mM NaN_3 for 2hr, determined from seedling growth tests and 100R and 200R gamma radiations were applied to 2000 seed samples of each genotype. Screening of the M_2 generation revealed that the mutagenic treatments induced morphological, physiological and biochemical changes in the genotypes. A spectrum of mutations which included variants with respect to anthocyanin pigmentation, leaf morphology, maturity date, male sterility and insect pest resistance qualities were observed. Lines with significant increases in yield parameters such as number of seeds per pod, peduncles per plant, 100 seed weight and seed storage proteins were selected.

Key words: *Vigna unguiculata,* cowpea, induced mutations, mutagens, varieties.

Cowpea is one of the most important pulse crops in tropical Africa. The seeds are a major source of dietary protein in most developing countries (Duke 1990). Induced mutation breeding which has been recognized as a valuable supplement to conventional breeding in crop improvement has been least applied in grain legumes. For example, only eight out of over 1000 improved mutant varieties of different crops released up to 1989 in over 48 countries were cowpeas (Micke *et al.* 1990).

In Kenya, Pathak (1991) obtained two aphid resistant mutants of cowpea from an M_2 population of susceptible seeds irradiated with 20kr of gamma rays. Other attributes of the improved lines include increased pod length, semi-erect plant habit and higher grain yield. In Nigeria, crop breeding through induced mutation is limited (Odeigah 1991). The present investigation was undertaken to induce viable mutations in quantitative and qualitative traits which could be utilized directly or introduced into our cowpea improvement program. We report here some of our results on the use of radiation and chemically induced mutations for crop improvement in cowpea.

MATERIALS AND METHODS

Plant Materials: Mature dry seed samples of two IITA cowpea varieties; IT84E-124 and Vita 7 were obtained from the Grain Legume Improvement Program (GLIP), International

Institute of Tropical Agriculture (I.I.T.A.)Ibadan, Nigeria. The two cultivars have good grain yield potential but are susceptible to most cowpea insect pests. IT84E-124 is erect, determinate with ovate leaves and have big seeds with rough seed coats. Vita 7 is spreading, indeterminate with hastate leaves and have small seeds with smooth seed coats.

Mutagenic Treatment And Seedling Growth Tests: Three mutagens, ^{60}Co gamma rays, ethyl methane sulfonate (EMS) and sodium azide (NaN_3), were used. Seeds were treated with 100R and 200R of gamma rays from a ^{60}Co source at the Seibersdorf Laboratory of the International Atomic Energy Agency (I.A.E.A..) Austria. For EMS and sodium azide treatments, seeds were presoaked in distilled water for 1 to 2 hr. at room temperature and then transferred to aqueous solutions of 5,10,25, and 40mM EMS for 3,6,9, and 12 hr respectively or to 0.01,0.1, 1.0 and 10mM NaN_3 for 2 and 4 hr respectively. The seeds were then washed in running tap water for 1 hr and transferred to petri dishes containing two layers of moist filter paper for germination. Five petri dishes of five seeds per treatment were planted and percentage germination and seedling variations for each treatment were subsequently determined.

Generation Of The M_1 And M_2 Populations: 2000 seeds of each variety were treated with 100R and 200R gamma rays, 10mM EMS for 6hr, 0.1mM and 1.0mM NaN_3 for 2hr. These doses were chosen because previous germination assays indicated that these doses may be suitable for mutation breeding. After postwashing, the seeds were planted (25 by 50cm) on I.I.T.A. fields. Plots were cultivated as necessary and protected with Sherpa Plus insecticide. At least three pods from each M_1 plant were harvested and bulked to form the M_2 generation.

2000 seeds per treatment per cultivar were planted with insecticide protection and observations on seedling emergence, survival and morphological changes were noted. Data on yield parameters like number of peduncles per plant, pods per plant, seeds per pod, seed weight and plant height were recorded and compared statistically with the controls using the Student's t test. Plants of interest were harvested individually, and the rest bulked.

Extraction And Determination Of Seed Protein Content Of M_3 Selected Plants: Two seeds from the same plant of each M_3 selection listed in Table 3 were separately dehulled and ground in a mortar and the extracts were defatted by washing with three changes of cold acetone for 4 to 6hr. The acetone was removed by filtration and the extracts were air-dried at room temperature. The proteins from the defatted meal were precipitated with 10% trichloroacetic acid and recovered by centrifugation at 5000rpm for 30 min at 4°C. The protein content was then determined colorimetrically according to the method of Lowry *et al* (1951) using bovine serum albumin (BSA) as standard.

Insect Pest Resistance Screening: The M_3 generation was grown without insecticide protection. 347 plants selected across the different populations as having the least aphid populations were screened for aphid resistance qualities. They were screened in the greenhouse according to the method of Jackai and Singh (1988). Briefly, the test materials along with a susceptible check were planted in single rows in wooden trays filled with soil. About 10 days after planting, each plant was infested with five fourth instar nymphs using a camel-hair brush. Infested trays were transferred into cages with fine saran mesh in the greenhouse. About two weeks after infestation, plants were assessed for vigour. Seeds of some lines were also screened for bruchid resistance following the procedure of Jackai and Singh (1988). Briefly, 20 seeds of each test material were placed in small plastic boxes with two pairs of day-old adult bruchids to oviposit for 24 hours. Five days after infestation, eggs laid per seed lot were counted and starting from 25 days after infestation, emerging adults were counted and removed until no further emergence from the susceptible control. Suitability index which is

$$= \log \frac{\text{percentage adult emergence}}{\text{mean development time}}$$

was used to estimate the resistance status of test materials.

M_4 Generation: 24 different mutant plants from among the M_3 generation selections were advanced with their parents in a randomized block design with four replications. Each line was grown in 2 rows of 2.5m.

RESULTS

With respect to the germination seedling growth test, ^{60}Co gamma radiation produced a number of physiological effects including reduced and late germination in comparison to the controls in both cultivars. 200R produced more effects that 100R. The response of the seeds to EMS and NaN_3 treatments at different doses and durations is shown in Fig 1a and b respectively. For EMS treatment, a low dose (5mM) gave high germinability but little or no variability while a high dose (40mM) gave good morphological variations but low seed survival or germinability. For all doses, long duration of exposure led to significant decrease in germinability. Long exposure to a high dose had a cytotoxic effect leading to lack of germination of the seeds. The overall data revealed that a dose of 10mM for a duration of 6hr gave good germination and morphological variations and is thus appropriate for mutation breeding (fig. 1a). The overall response of the varieties to NaN_3 treatment was similar to that of EMS. Doses as low as 0.1mM for 2hr duration resulted in more than 50% germination (fig. 1b) and variations in the plant morphology. Although the responses of the two varieties to EMS and NaN_3 treatments were similar, more pronounced effects were observed in IT84E-124 (see fig. 1a and b).

The three mutagens (^{60}Co gamma radiation, EMS and NaN_3) at the doses used induced a broad spectrum of mutations in the M_2 plants of the two varieties. The changes observed were morphological, physiological and biochemical. Both desirable and non-desirable effects were observed. Plants with branched peduncles, anthocyanin pigmentation in the pods and stem, changes in leaf and flower shape and colour, male sterility, early maturity and aphid and bruchid resistance were recognized across the different treatments. The bruchid resistant selection had percent adult emergence and suitability indices of 30.56 and 1.193 respectively compared to 45.46 and 1.715 of the control. Non-selectable mutant (plants with least/poor agronomic values) observed include plants with stunted growth, twining stem and spreading growth habit.

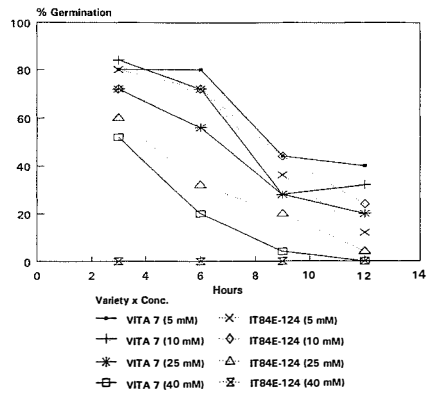

Fig 1a: Germination % of IT84E-124 & VITA 7 soaked in different conc. of EMS.

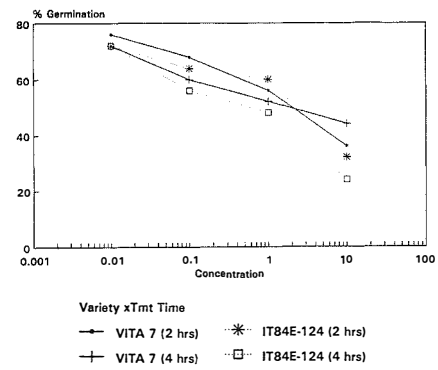

Fig 1b: Germination % of IT84E-124 & VITA 7 in conc. of NaN_3 for 2 & 4 hours.

Tables 1 and 2 show the yields and the corresponding increases and decreases of M_2 generation yield components induced by the various treatments in var. IT84E-124 and var. Vita 7 with respect to the controls. Varietal differences

were noticed in the two varieties. EMS treatment and 100R gamma irradiation did not effect any improvement in the yield parameters of IT84E-124 (Table 1). For Vita 7, 100R gamma irradiation made positive changes in peduncles and pods per plant and the flowering date, while EMS affected the 100 seed weight and seeds per pod positively (Table 2). 200R gamma irradiation and the two NaN_3 (0.1 and 1.0mM) treatments induced significant improvements in the plant height, peduncles per plant and pods per plant in both IT84E-124 and Vita 7 (see tables 1 and 2).

The seed protein contents (mg/ml) of some of the selected M_3 mutants are shown in Table 3. As can be seen, some mutants have higher values while some have lower values compared to the original or parent lines. In IT84E-124 the highest increase of 13.3% was found in line AR-3 which is an aphid resistant line obtained by 1.0mM NaN_3 treatment while the highest increase in Vita 7 was 13.64% found in line 7P which showed no visible morphological or physiological change. Tables 4 and 5 show the mean yield of different parameters in the two varieties and their M_4 mutants. The tables show variations in all parameters studied. For example the aphid resistant lines of IT84E-124 (AR-1, AR-2, AR-3) produced seeds per pod or higher 100-seed weight than the control. Also the Vita 7 mutant, lanceolate leaf, LL, produced more peduncles and pods/plants than the parent.

DISCUSSION

Development of improved plant cultivars is restricted by limited genetic resources. Mutation induction could create additional genetic variability to supplement conventional crop breeding. NaN_3, EMS and ^{60}Co gamma radiation induced genetic variability in the two varieties, IT84E-124 and Vita 7. Lines were produced with agronomically useful traits as well as lines with traits that can be used only as genetic markers. Responses of the two cultivars to the different treatments differ. Fehr (1987) reported that differences exist among species and among genotypes within a species for sensitivity to mutagen treatment. It has been shown in the pulse crop, lentil *(Lens culinaris)* that the mutation spectrum depends greatly on the genotype and the type and dose of mutagens used (Shaikh and Begum 1991). Similar findings were observed in the two cowpea varieties (Vita 7 and IT84E-124) which are products of different genetic crosses and differ in a number of morphological and physiological characters.

Use of induced mutations for obtaining early maturing cultivars has been a frequent breeding objective (Micke 1979). The early maturing selections could serve as candidates for short season cowpea. Ashraf (1985) found early maturing cowpea useful in areas with short rainfall and as relay crops in rice paddies. Pulses generally have yield per hectare lower than cereals. Plant with increases in yield parameters and total protein content have a promising possibility of improving total protein yield per hectare. Similar findings have been reported in pea mutant (Jaranowski and Micke 1985). Increases in a polygenic character like yield could result from changes in simply inherited traits (Micke *et al* 1990) or mutations at the structural loci (Evans 1987).

We are currently studying the three aphid resistant lines and a bruchid resistant line that we selected. These and plants with anthocyanin pigmentation in pods and stems which are less attractive to insects (Thorstenison 1980) could find application in integrated pest management program. Cowpea, a basically self-fertilizing crop could benefit from genetic male sterility in the improvement of the low level of outcrossing found in the crop. Studies of the different male sterile lines and the associated floral aberrations gave an insight into the crop's genome (Odeigah *et al.* 1996).

This study, as well as many previous ones, in which agronomically useful mutants and those that could serve only as genetic markers have been produced through mutagenesis indicate that induced mutation breeding is a valid and effective crop breeding method even in cowpea.

TABLE 1

Yield of M_2IT84E-124 exposed to different doses of the three mutagens+

Treatment	100R gamma rays	200R gamma rays	10mMx6hr.EMS	0.1$NaN_3$2hr.	1.0NaNx2hr.	Control
Yield Parameters						
Peduncles/Plant	11.79±4.59 (-7.53)	13.97±4.13 (9.56)	10.22±2.17 (-19.84)	0.60±2.82 (-16.86)	21.29±10.36* (66.98)	12.75±2.77
Pods/plant	17.27±8.36 (-3.21)	18.59±5.64 (4.26)	11.68±3.42 (-34.49)	27.81±15.70* (55.97)	34.86±17.09* (95.51)	17.83±4.16
Plant height (cm)	16.60±3.59 (-3.21)	17.73±3.15 (3.38)	11.31±1.85 (-34.05)	36.49±15.40* (112.77)	29.26±36.17* (70.49)	17.15±1.41
100 seed weight (g)	13.35±0.36 (-0.37)	13.85±0.55* (3.36)	12.70±0.71 (-5.22)	11.75±0.35 (-12.31)	10.80±0.34 (-19.40)	13.40±0.21
Seeds/Pod	9.11±3.43 (8-72)	9.00±2.38 (-9.82)	5.78±2.29 (42.08)	10.65±2.91 (6.71)	11.73±3.51* (17.53)	9.98±2.46
Germination percent	72.80 (1.11)	48.70 (-32.36)	62.10 (-13.75)	45.80 (-36.39)	43.60 (-39.44)	72.00
50% flowering	32 (8.75)	41 (-17.14)	35 (0.0)	36 (2.86)	36 (2.86)	35

+Values are sample means (S.D. (% increase or decrease)
*Better than control, $p<0.05$

TABLE 2

Yield of M_2 Vita 7 exposed to different doses of the three mutagens+

Treatment	100R gamma rays	200R gamma rays	10mMx6hr.EMS	0.1$NaN_3$2hr.	1.0NaNx2hr.	Control
Yield Parameters						
Peduncles/Plant	19.19±5.63* (45.60)	16.49±4.46 (25.11)	11.47±2.74 (-12.97)	35.88±11.78* (172.23)	42.60±12.99* (223.22)	13.18±3.97
Pods/plant	22.51±7.38* (47.32)	20.59±7.90* (34.16)	12.19±3.48 (-20.22)	34.74±14.71* (125.59)	36.98±15.10* (142.02)	15.28±4.87
Plant height (cm)	25.26±10.07 (14.20)	28.47±10.53* (28.71)	21.15±4.55 (-4.39)	138.74±48.32 (527.22)	125.0±52.30* (465.10)	22.12±1.63
100 seeds weight (g)	13.23±0.43 (0.23)	13.20±0.48 (0.00)	18.83±0.29 (42.65)	12.15±0.41 (-7.95)	12.60±0.21 (-4.55)	13.20±0.46
Seeds/Pod	11.59±3.61 (-0.7)	10.14±2.99 (-12.59)	12.76±2.05 (10.00)	10.46±2.48 (-8.28)	10.60±2.79 (-9.48)	11.60±2.12
Germination percent	67.70 (5.78))	31.00 (-51.56)	57.30 (-10.47)	50.45 (-21.17)	51.85 (-18.94)	64.00
50% flowering	39* (8.75)	47 (-17.14)	41* (0.0)	45 (2.86)	46 (2.86)	49

+Values are sample means±S.D. (% increase or decrease)
*Better than control, $p<0.05$

TABLE 3

Mean protein content (mg/ml) of some M_3 selected lines

Line	Mutation Spectrum	Protein Content
	IT84E-124	
P124	Erect, ovate leaves, brown rough seed, white flowers, (parent) IT84E-124	110.0±.0339
SS	Smooth seed	115.0±.0707
4P-1	Like the parent	100.0±.0530
SM	Very small seed	120.0±.0884
SL	Smooth and light brown seed	87.5±.0494
AR-1	Aphid resistant, small, slightly speckled seed	117.5±.0707
AR-2	Aphid resistant, robust and big seed	115.0±.0707
AR-3	Aphid resistant	125.0±.0884
CS-1	Cream seeds, flowers with anthocyanin	115.0±.0707
RS	Very rough seed	110.0±.0707
SSA	Smooth seeds, anthocyanin in stem and peduncles	111.25±0.0707
BS	Big seeds	120.0±0.0849
	Vita 7	
PV7	Semi erect, hastate leaves, smooth brown seeds, anthocyanin in flowers, (parent Vita 7)	112.5±0.0686
GA	Giant, anthocyanin in pods, dark brown seeds	112.5±0.0636
EF-2	Early flowering	77.5±0.1767
MS	Maroon seeds	92.5±0.0636
7P	Like the parent	127.5±0.0707
LBS	Light brown seeds	115.75±0.0884
GLF-1	Giant, anthocyanin, late flowering and reduced fertility	126.75±0.0955
LL	Lanceolate leaves, late flowering and reduced fertility	117.5±0.0778

TABLE 4

Mean Performance of yield Parameters of some M_4 IT84E-124 Mutant Lines[1].

Lines	Peduncle/Plant	Pods/Plant	Plant heigh (cm)	Seeds/Pod	100 Seed Weight (gm)	Germination %	50% Flowering
P124(Parent)	12.00±2.40	8.20±3.81	17.12±0.25	10.14±0.13	13.13±0.42	87.5	41
AR-1	13.50±1.07	19.00±5.95	16.67±0.15	10.08±0.20	13.80±0.14**	82.5	41
AR-2	10.50±3.05	17.90±3.98	18.65±0.28**	12.03±0.15**	12.70±0.21	90	39
AR-3	13.70±2.31	18.12±4.13	17.60±0.90*	10.68±0.10**	13.00±0.29	87.5	38
MS-1	10.70±1.76	16.10±6.58	16.83±0.21	9.02±0.17	12.60±0.21	92.5	38
MS-2	13.10±2.17*	21.10±3.36*	16.24±0.61	9.12±0.18	12.90±0.48	80	35
4P-1	12.90±2.70	16.30±2.23	18.10±0.13**	10.16±0.13*	13.40±0.41	85	40
4P-2	11.60±1.69	17.80±1.37	17.95±0.40**	11.62±0.19**	13.10±0.43*	90	38
4P-3	11.80±2.05	19.30±4.01	17.80±0.60**	10.10±0.13	12.90±0.27	85	38
4P-4	11.80±1.60	17.00±3.43	18.61±0.24**	10.60±0.17**	13.00±0.33	90	38

[1]Values are sample means ±SD
*Better than control, P<0.05
**Better than Control, P<0.01

TABLE 5

Mean Performance of yield parameters of some M_4 Vita 7 mutant lines.

Lines	Peduncle/Plant	Pedundes/Plant	Plant heigh (cm)	Seeds/Pod	100 Seed Weight (gm)	Germination	50% Flowering
Pv7(Parent)	19.75±2.06	20.75±1.71	25.80±2.37	12.36±0.56	11.60±1.45	82.50	44
7P-1	18.50±2.51	21.75±1.89	25.60±3.24	12.75±1.17	10.65±1.35	78.75	44
7P-2	17.00±0.825	19.25±1.26	32.70±1.94*	12.60±0.64	11.37±1.50	82.50	43
7P-3	18.75±3.30	26.25±1.56*	27.73±1.37	13.26±0.65	11.12±0.84	85.00	46
EF-1	21.50±2.14	20.502.38	28.13±2.99	12.70±0.48	12.01±2.18	90.00	41
LF	14.25±2.22	18.50±2.38	29.45±2.26	15.45±1.01*	10.80±1.60	75.00	51
AP-1	20.75±2.75	21.75±1.71	22.18±1.87	12.57±0.68	11.58±1.48	91.25	43
AP-2	16.75±2.63	23.25±2.50	29.08±2.99	12.76±0.67	10.46±2.38	78.75	43
CS-2	20.75±3.86	20.50±1.29	28.78±1.69	12.45±0.76	11.73±1.93	80.00	45
MS	19.25±2.75	21.75±2.36	24.78±2.17	13.70±0.88	10.93±0.75	82.50	47
7P-4	16.25±3.27	21.25±2.22	25.55±0.93	12.17±0.40	10.46±1.41	88.75	43
DbS	18.00±1.64	16.25±1.77	30.58±1.61	12.57±0.40	10.42±0.65	86.25	43
GLF-1	18.75±2.87	21.50±1.29	54.68±4.41**	12.40±0.52	13.80±0.93*	85.00	47
TS	19.00±2.45	19.00±1.15	28.03±1.70	12.25±0.57	11.32±1.85	93.75	44
LL	25.50±2.40*	29.50±2.87*	39.75±2.03**	12.66±0.18	10.50±0.88	82.50	45
EF-2	19.25±2.75	18.25±1.06	30.40±2.09	11.94±0.32	9.60±2.48	75.00	41

[1]Values are sample means ±SD
*Better than control, P<0.05
**Better than control, P<0.01

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