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Original Article

Optimizing induced mutation technique for the improvement of agronomic traits in pigeon pea [Cajanus cajan (L.) Millsp.] landraces

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Abstract

Creation of variability through induced mutation is cardinal in genotype selection. However, the stability of the mutants in the subsequent generations is of utmost importance for improvement. This current research was aimed at evaluating the performance of pigeon pea landraces following amiprophos methyl (APM) treatment. Thirty seeds each of two varieties of pigeon pea (brown "Fiofio", white "Fiofio") were soaked in 0, 4, 6 and 8 ppm amiprophos methyl (APM) for 48 hours. After the first growing season (2010/2011), mutant seeds were harvested and replanted in the next growing season (2011/2012). The result from the parental generation showed that the brown "Fiofio" variety grew taller (286.1±1.63cm), produced more branches plant^{-1} (18.25±0.25), had broader leaf area plant^{-1} (86.12±2.29cm^2) and increased petiole length plant^{-1} (4.58±0.23cm) while the white "Fiofio" produced more leaves plant^{-1} (337.5±1.04) and seed yield plant^{-1} (452.5±1.04). Plants raised from the M_1 mutant seeds of white "Fiofio" variety produced more pods plant^{-1} (267.8±1.93pods) and had increased seed yield plant^{-1} (1344± seeds) with increased weight of 100-seeds (18.12±0.11g). However, plants raised from brown "Fiofio" produced more number of leaves plant^{-1} (2865±2.73), had taller plants (255.2±1.77cm) with increased days to 50% flowering and maturity (196; 180 days, respectively). The treatment also caused high phenotypic and genotypic variances; especially in the M_1 generation. Explicitly, there were obvious positive significant effects of APM treatment on pigeon pea, especially the white variety at 4 ppm. M_1 generation progenies performed better than their parental counterparts in yield traits, including seed yield. The implication is that M_1 seeds can be advanced to M_2 generation where genetic blueprint will be released through segregation.

Key words: Amiprophos methyl, induced mutation, improvement, pigeon pea

Otimização técnica de indução de mutação para a melhoria de características agronômicas em guandu [Cajanus cajan (L.) Millsp.] Landraces

Resumo

A criação de variabilidade através de mutação induzida é cardeal em seleção de genótipos. No entanto, a estabilidade dos mutantes nas gerações subsequentes é de extrema importância para a melhoria. Esta pesquisa teve como objetivo principal avaliar o desempenho de cultivares de guandu, tratamentos com amiprophos metil (APM). Trinta sementes cada uma das duas variedades de guandu (marrom "Fiofio", branco "Fiofio") foram embebidas em 0, 4, 6 e 8 ppm amiprophos metil (APM) por 48 horas. Após a primeira estação de crescimento (2010/2011), sementes mutantes foram colhidas e replantadas na safra seguinte (2011/2012). O resultado da geração dos pais mostrou que a variedade marrom "Fiofio" cresceu mais alto (286,1 ± 1,63 centímetros), produzido mais ramos planta^{-1} (18,25 ± 0,25), teve mais amplo de área foliar da planta^{-1} (86,12 ± 2.29cm2) e aumento pecíolo comprimento planta^{-1} (4,58 ± 0,23 centímetros), enquanto o branco "Fiofio" produziu mais folhas planta^{-1} (337,5 ± 1,04) e rendimento de sementes de plantas^{-1} (452,5 ± 1,04). Plantas levantadas a partir da M1 sementes mutantes de branco "Fiofio" variedade produziu mais vagens planta^{-1} (267,8 ± 1.93pods) e aumentou o rendimento de grãos da planta^{-1} (1.344 sementes ±) com o aumento do peso de 100 sementes (18,12 ± 0,11 g). No entanto, as plantas criadas a partir de marrom "Fiofio" produziu o maior número de folhas planta^{-1} (2,865 ± 2,73), tinha plantas mais altas (255,2 ± 1,77 centímetros) com o aumento dias a 50% de floração e maturação (196; 180 dias, respectivamente). O tratamento também causou alta fenotípica e variância genotípica; especialmente na geração M1. Explicitamente, não houve efeitos significativos positivos óbvios de tratamento APM em guandu, especialmente a variedade branca a 4 ppm. Progênies geração M1 desempenho melhor do que os seus homólogos dos pais em caracteres de produção, incluindo a produção de sementes. A implicação é que as sementes M1 pode ser avançado para geração M2 onde código genético será lançado pela segregação.

Palavras-chave: *Amiprophos metilo*, mutação induzida, melhoria, guandu

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Introduction

For sustainable food security in the sub-Saharan African countries, particularly Nigeria, the need for crop development and improvement, especially landraces should not be over-emphasized. Mutation induction on crop plants has been reported to create variability, which is a prerequisite for crop improvement (Mahandjiev et al., 2001; Ciftci et al., 2006; Boureima et al., 2009; Udensi et al., 2012d; 2013).

Different authors have reported the use of different mutagenic agents for the improvement of specific crops and highlighted significant effects (Xiuzher, 1994; Rabie et al., 1996; Stoeva & Bieneva, 2001; Ciftci et al., 2006; Khan & Al-Qurainy, 2009; Brisibe et al., 2011; Udensi et al., 2012c, d). Several researchers have reported polyploidy induction following mutagenic treatments and have affirmed to improve agronomic traits in crops (Takamura & Miyajima et al., 1986; Yamaguchi, 1989; Solo'veva, 1990; Keeler & Davis, 1999; Carvalho et al., 1999; Brisibe et al., 2011; Udensi et al., 2011c; 2013,).

Amiprophos methyl (APM) is an example of a phosphoroamidates herbicide, which has been used intensively in agriculture. This notwithstanding, it has been reported to effect shoot and root elongation and development severely, causing both shoots and roots to be stunted with a characteristic swollen tip (Anthony & Hussey, 1999). APM action is by binding to the a,b-tubulin dimer site, thus inhibiting microtubule polymerization. This however, prevents the formation of chromatic fuses and induces separation of the metaphasic chromosomes (Blume et al., 2003).

Legumes have been reported to have high nutritive values, especially protein with balanced amino acids profile (Tharanathan & Mahadevamma, 2003; De Almeida Costa et al., 2006; Udensi et al., 2011b). Specifically, pigeon pea [*Cajanus cajan* (L.) Millsp] landraces with high adaptive potential to environmental stresses (Joshi et al., 2009) is one of the legumes that concerted efforts should be geared towards improving given that worsening climatic conditions in the globe demands crops that have the intrinsic capacity to withstand these precarious situations (Udensi et al., 2011a; 2012a, b).

Worthy of note is the fact that inducing mutation that will lead to improve agronomical traits is one thing and it is yet another for the introgression of economic traits from parentals to the mutants in the subsequent generations to be favourable. It thus implies that the stability of these traits is very cardinal to successful mutagenesis. This current research is aimed that evaluating the performance of pigeon pea landraces following amiprophos methyl (APM) treatment and thereafter assess the stability of the M_1 mutants. Efforts were also made to compare the variability between the parents and M_1 mutants. This will aid in ascertaining the impact of APM in pigeon pea improvement.

Material and methods

Experiment 1: Studies on the parental generation

Seeds of two varieties of pigeon pea (brown "Fiofio", white "Fiofio") were obtained from the germplasm collection of Dr. Udensi, O. Ugorji at the University of Calabar, Nigeria. Thirty seeds were soaked in 50ml of each of the APM concentration, 0, 4, 6 and 8 ppm for 48 hours bringing the final volume to 60cm^3. Eight beds were made with a spacing of 2 meters between beds. The treated seeds were then sown on a plot of land measuring 12x12 meters using randomized complete block design in a 2x4 factorial layout with 10 replications. Three seeds per variety were sown in a hole of 4cm deep according to the method of (Center for New Crops and Plants Products, 2002). A spacing of 20x75cm was maintained between stands. This experiment was carried out in the University of Calabar Experimental Farm, Calabar, Nigeria, during the 2010-2011 growing season.

Experiment 2: Studies on the M_1 mutants

During the 2011/2012 growing season, M_1 seeds were obtained from experiment 1 and sown according to the methods of (Center for New Crops and Plants Products, 2002) while data were collected according to the method of Udensi et al. (2012a).

Data collection and analysis

After one month of planting in the two

experiments, percentage germination and days to seedling emergence were estimated. Other morphological traits such as plant height plant^{-1}, number of branches plant^{-1}, number of leaves plant^{-1}, leaf area plant^{-1}, internode length plant^{-1}, petiole length plant^{-1}, days to 50% flowering, number of flowers plant^{-1}, number of pods plant^{-1}, pod length, number of seeds pod^{-1}, days to 50% maturity, seed yield plant^{-1}, and 100-seed weight were also recorded at 6 months. For the estimation of the leaf area, the leaves were laid on a 1-cm grid (graph paper) and their outlines were traced. The numbers of square centimeters were calculated, including the partial square and multiplied by 0.1cm$^{2.}$ However, all partial squares that are less than half covered were excluded. The seed yield per plant was estimated by multiplying the average number of seeds per pod per plant and the average number of pod per plant (Udensi et al., 2012a). They were subjected to analysis of variance (ANOVA) using Predictive Analytics SoftWare (PASW), version 18.0. Genetic estimates such as phenotypic (δ^2p) and genotypic variances (δ^2g) were done by the method of (Uguru, 1998),

Results

Morphological and yield traits of parental generation of pigeon pea after amiprophos methyl (APM) treatment

Seeds of pigeon pea parental lines were exposed to amiprophos methyl and interesting observations were made. There were significant effects ($P < 0.05$) of APM treatment on the morphological and yield traits of the two varieties evaluated, which were concentration-dependent. It showed that plants derived from 4 and 8 ppm soaked seeds of brown Fiofio grew taller (286.1±1.63; 276.5±2.20); produced broader leaves (86.12±2.29; 84.99±1.87) and enhanced the number of branches (18.25±0.25). Though the plants derived from white Fiofio seeds produced more pods plant^{-1} (91.75±1.25), number of leaves plant^{-1} (337.5±1.04) and seed yield plant^{-1} (452.5±1.04), generally, brown variety performed better, especially on morphological traits (Table 1). Varietal and concentration means separation revealed also that brown "Fiofio" variety grew taller (270.44cm), produced more branches plant^{-1} (17.31), had broader leaf area plant^{-1} (83.37cm^2) and increased petiole length plant^{-1} (6.83cm) while the white "Fiofio" produced more leaves plant^{-1} (~310). Additionally, the germination percentage (~98 days) increased including the days to 50% flowering (183 days) for the brown variety while days to 50% maturity increased for white variety (~198 days) (Table 2).

The differentials observed in the variety notwithstanding, the concentration of APM used had a dose-dependent effect on the morphological traits. It revealed that increasing APM concentration caused increase in the height of the plant, leaf area, number of leaves and number of branches plant^{-1}. Specially, seeds exposed to 4 ppm APM produced more flowers, pod number and gave the highest seed yield plant^{-1} though it reduced days to 50% flowering and maturity, the trend was consistent (Table 3).

Morphological and yield traits of M_1 generation of pigeon pea after amiprophos methyl (APM) treatment

M_1 seeds were planted to assess their performances; it was observed that there were remarkable improvements in most traits, the variety notwithstanding. Plants raised from seeds of white Fiofio soaked in 4 ppm APM produced more flowers plant^{-1} (531.4±1.53), pod plan^{t-1} (267.8±1.93) and seed yield plant^{-1} (1344.0±2.48). Other trait such as number of leaves plant^{-1}, number of branches plant^{-1}, pod length competed favourably with plants raised from brown Fiofio at the same concentration 4 ppm. Separating the means based on varietal and concentration showed that plants raised from the M_1 seeds of white "Fiofio" variety produced more pods plant^{-1} (~157 pods) and had increase seed yield plant^{-1} (777 seeds) with increased weight of 100-seeds (17.32g). However, plants raised from brown "Fiofio" produced more number of leaves plant^{-1} (~2716), had taller plants (230.6cm) with increased days to 50% flowering and maturity (184; 176 days, respectively). Though there were no trend followed concerning APM effect on the evaluated traits, result revealed a dose-dependent effect. It was observed that there was stability of traits that were hitherto positively affected by 4 ppm APM treatment such as increased number of leaves (2568), number of flowers (494), number of pods (192) and seed yield plant^{-1} (946) (Figure 1). The lengths of the petiole and internode increased with the days to 50% flowering though it reduced with increasing mutagen concentration (Tables 4 & 5).

Table 1. Effect of amiprophos methyl on yield and yield-related traits in pigeon pea parental generation

Morphological/ yield traits	Brown fiofio				White Fiofio			
	Concentration of amiprophos methyl used (ppm)							
	0	4	6	8	0	4	6	8
Days to seedling emergence	4.8±0.21b	4.6±0.2b	4.25±0.22b	5.53±0.43a	4.75±0.23b	4.0±0.02b	4.65±0.28ab	5.2±0.31ab
% Seed germination	85.3±1.1c	99.98±0.2a	99.99±0.3^{a}	94.5±0.01b	89.75±0.5c	88.50±0.3c	88.5±0.25c	95.8±0.3b
Plant height plant^{-1}	254.7±2.3bc	286.1±1.63a	264.5±2.10b	276.5±2.20a	231.1±1.75d	237.0±1.4d	249.6±1.12c	250.2±1.56c
Number of leaves plant^{-1}	284±1.47e	264.0±0.05f	282.75±0.085e	318.75±2.14b	314±1.58c	337.5±1.04a	305.5±2.06d	283.5±0.65e
Number of branches plant^{-1}	16.5±0.65b	18.25±0,25a	16.0±0.41b	18.5±0.5a	14.5±1.13c	10.75±0.25e	12.75±0.48d	14.51±0.29c
Leaf area plant^{-1}	84.94±1.12a	86.115±2.29a	77.44±1.68b	84.99±1.87a	55.06±1.00e	57.41±1.14e	61.13±1.07d	67.10±1.41c
Internode length	7.55±1.06a	6.175±0.72ab	5.0±0.83bc	5.15±0.08bc	5.625±0.25bc	4.45±0.26c	5.2±0.08bc	4.45±0.09c
Petiole length	4.45±0.27a	4.58±0.23a	3.65±0.18b	4.75±0.15a	3.0±0.04c	2.93±0.11c	3.75±0.21b	3.75±0.32b
Days to 50%flowering	178.3±1.75b	195.50±1.96a	167.0±1.78c	191.75±1.31a	162.5±1.04cd	163.75±1.25c	158.0±2.04d	152.0±2.17e
Number of flowers	84.75±1.11d	112.75±0.85c	173.75±1.38a	85.25±1.11d	132.5±1.32b	122.8±0.85bc	66.5±1.04e	116.8±0.85c
Days to 50% maturity	182.5±1.19d	189.5±1.04bc	183.5±1.53d	183.5±1.19d	203.25±0.85a	205.25±0.85^{a}	189±1.63c	192.5±0.63b
Pod length	6.13±0.14a	6.0±0.08a	6.2±0.15a	7.65±0.65a	6.35±0.65a	6.075±0.09a	5.53±0.13a	5.023±0.37a
Number of pod per plant	69.25±2.06c	67.5±1.85c	90.25±0.63a	51.25±1.11d	77.75±1.75b	91.75±1.25a	44.00±1.68e	78.25±1.65b
Number of seeds pod^{-1}	4.75±0.25a	5.25±0.25a	4.5±0.65a	6±0.41a	5.0±0.41a	4.75±0.48a	5.25±0.23a	5.1±0.41a
Seed yield plant^{-1}	335.3±2.25c	342.5±5.20c	415±20.62b	289±13.53d	387.5±6.61b	452.5±1.04a	222.5±3.23e	394.0±1.96b
100 seed weight	13.4±0.2abc	15.11±0.39a	11.96±0.84c	14.22±0.63ab	12.27±0.91bc	12.11±0.76c	11.9±0.10c	12.5±0.68bc

Means followed with the same case letter along horizontal array indicate no significant effect ($P > 0.05$)

Table 2. Effect of amiprophos methyl on yield and yield-related traits in pigeon pea M_1 generation

Morphological/ yield traits	Brown fiofio				White fiofio			
	Concentration of amiprophos methyl used (ppm)							
	0	4	6	8	0	4	6	8
Days to seedling emergence	4.5±0.29b	4.0±0b	4.25±0.25b	5.73±0.63a	4.25±0.25b	4.0±0.01b	4.75±0.48ab	5.0±0.41ab
% Seed germination	60.0±0.04a	52.0±0.82a	48.1±0.69a	32.0±0.8ab	44.2±0.04a	28.3±0.89b	28.4±0.89b	24.5±0.08b
Plant height plant^{-1}	244.6±1.57b	255.2±1.77a	210.0±1.70dd	212.6±1.50d	230.0±1.1c	214.8±1.77d	234.8±1.77c	198.6±1.44e
Number of leaves plant^{-1}	2846.4±1.39a	2565.2±1.3b	2865.4±2.73a	2587.8±1.7b	2134±4.2c	2570.4±2.3b	2002.8±1.4d	1686.6±2.7e
Numbers of branches plant^{-1}	24.0±1.73ab	21.8±2.18ab	21.0±0.2b	26.8±1.28a	25.4±2.04ab	20.4±1.78b	22.2±1.53ab	22.0±1.92ab
Internode length	7.22±064	10.0±0.17a	7.7±0.34b	7.38±0.15b	8.68±1.00ab	7.78±0.60b	7.72±0.44b	5.50±0.39c
Petiole length	5.38±0.36cd	8.46±0.17a	6.78±0.25bc	5.94±0.32cd	4.88±0.27d	7.84±0.94ab	5.04±0.52d	8.24±1.08ab
Days to 50% flowering	178.5±1.55b	195.5±1.55a	167.5±1.55c	192.25±0.2a	163.5±1.04c	164.5±1.55c	159±1.87d	153.25±2.2e
Number of flowers plant^{-1}	339±0.59c	456.8±1.13b	326±2.71c	212.6±2.89e	313.6±4.2cd	531.4±7.53a	328.8±4.12c	281.2±1.37d
Days to 50% maturity	179±4.83a	179.8±2.22a	175.6±4.47a	171.4±0.8a	153±1.64b	155±0.84b	166±2.93a	171.8±1.11a
Pod length	6.9±0.06b	7.86±0.09a	7.48±0.21a	7.46±0.20a	7.44±0.22a	6.86±0.16b	6.94±0.19b	5.9±10,13c
Numbers of seeds pod^{-1}	4.4±0.25b	5.2±0.2a	4.0±0.32c	5.1±0.32a	3.4±0.2c	5.1±0.32^{a}	4.8±0.2a	4.4±0.25b
Number of pods plant^{-1}	52±2.81f	142.4±1.3c	104±1.06d	78.6±2.79e	29.8±1.66g	267.8±1.93a	242.4±3.61b	86.6±.162e
Seed yield plant^{-1}	260±9.35e	648.8±3.2c	441.6±1.9d	398±16.56d	98.4±2.25f	1344±7.48^{a}	1244±2.5b	421.6±0.5d
100-seed weight	15.3±1.14a	10.74±0.28d	10.82±0.19d	12.54±0.20c	17.22±0.27a	18.12±0.11a	16.9±0.43a	17.3±0.23a

Means followed with the same case letter along horizontal array indicate no significant effect (P > 0.05)

Table 3. Varietal mean separation of yield and yield related morphological traits of parental lines that were treated with AMP

Morphological traits	Brown "Fiofio"	White "Fiofio"
Days to seedling emergence (days)	4.38±0.18a	4.37±0.13a
Percentage seed germination (%)	97.69±1.96a	91.75±1.28b
Plant height plant^{-1} (cm)	270.44±1.45a	241.96±2.23b
Numbers of leaves plant^{-1}	287.56±2.12b	310.13±0.59a
Number of branches plant^{-1}	17.31±0.35a	13.13±0.43b
Leaf area plant^{-1} (cm^2)	83.37±1.19a	60.17±1.29b
Internode length plant^{-1} (cm)	5.97±0.40a	4.95±0.16a
Petiole length plant^{-1} (cm)	6.83±2.48a	3.36±0.14b
Days to 50% Flowering (days)	183.00±2.34a	159.13±1.39b
Number of flowers plant^{-1}	114.13±1.89a	109.63±3.02a
Number of pods plant^{-1}	69.56±1.31a	72.94±1.24a
Pod length plant^{-1} (cm)	6.49±0.24a	6.01±0.19a
Number of seed pod^{-1}	5.13±0.24a	5.00±0.18a
Days to 50% Maturity (days)	184.75±0.91b	197.5±1.84a
Seed yield plant^{-1}	345.44±4.32a	364.13±2.36a
100-seed weight (g)	13.67±0.41a	12.2±0.35a

Means followed with the same case letter along horizontal array indicate no significant effect ($P > 0.05$)

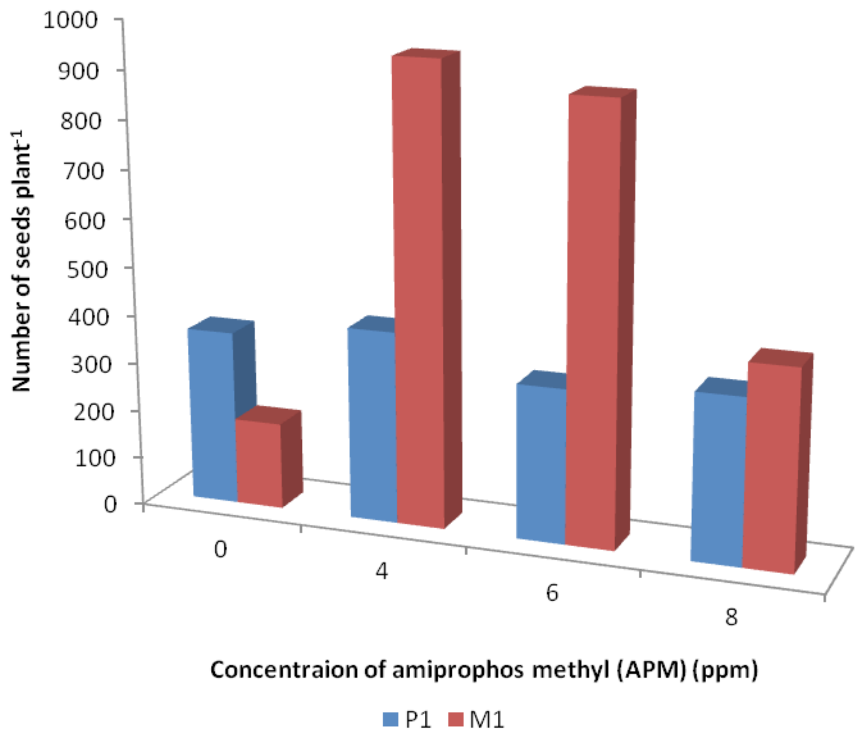

Figure 1. Concentration effect on seed yield plant^{-1} in the parental (P_1) and first mutant generations (M_1)

Table 4. Varietal means separation of yield and yield related morphological traits obtained from M_1 generation

Morphological traits	Brown	White
Percentage seed germination (days)	48.0±0.30^{a}	31.0±0.70^{b}
Days to seedling emergence (%)	4.63±0.24a	4.50±0.18a
Plant height plant^{-1} (cm)	230.60±4.58^{a}	219.55±3.34^{b}
Number of leaves plant^{-1}	2716.2±37.2^{a}	2098.45±2.8^{b}
Number of branches plant^{-1}	23.4±0.99^{a}	22.5±0.94^{a}
Internode length plant^{-1} (cm)	8.08±0.312^{a}	7.42±0.40a
Petiole length plant^{-1} (cm)	6.64±0.29^{a}	6.5±0.50^{a}
Days to 50% flowering (days)	183.5±2.94a	160.06±1.38b
Number of flowers plant^{-1}	333.62±0.30^{a}	363.75±22.76^{a}
Days to 50% maturity (days)	176.45±1.78^{a}	161.45±1.97^{b}
Number of pod plant^{-1}	94.25±0.56^{b}	156.65±3.32^{a}
Pod length plant^{-1} (cm)	7.43±0.11^{a}	6.79±0.15^{a}
Number of seeds pod^{-1}	4.65±0.17^{a}	4.40±0.18^{a}
Seed yield plant^{-1}	437.1±0.38^{b}	777.0±1.22^{a}
100-seed weight (g)	12.67±0.63^{b}	17.32±0.18^{a}

Means followed with the same case letter along horizontal array indicate no significant effect (P > 0.05)

Table 5. Mean separation of yield and yield-related morphological traits of M_1 generation in respect to treatments

Morphological traits	Concentrations of amiprophos methyl (ppm)			
	Control	4	6	8
Percentage seed germination (days)	52.0±0.23a	40.0±0.54^{ab}	38.0±0.52^{b}	28.0±0.25^{c}
Days to seedling emergence (%)	4.38±0.13ab	4.00±0.01b	4.5±0.19ab	5.38±0.27a
Plant height plant^{-1} (cm)	237.3±2.67^{a}	235.0±0.84^{a}	222.4±4.29^{b}	205.6±2.53^{c}
Number of leaves plant^{-1}	2490.2±0.79^{b}	2567.8±0.31^{a}	2434.1±3.78^{c}	2137.2±0.02^{d}
Number of branches plant^{-1}	24.7±1.28^{a}	21.1±1.35^{a}	21.6±1.20^{a}	24.4±1.35^{a}
Internode length plant^{-1} (cm)	7.95±0.61^{ab}	8.89±0.47a	7.71±0.27^{ab}	6.44±0.37c
Petiole length plant^{-1} (cm)	5.13±0.22^{c}	8.15±0.46^{a}	5.91±0.39^{bc}	7.09±0.65^{ab}
Days to 50% flowering (days)	171.0±2.96b	180.0±2.34a	163.38±2.02c	172.72±1.45b
Number of flowers plant^{-1}	326.3±0.15^{b}	494.1±3.95^{a}	327.4±2.21^{b}	246.9±2.13^{c}
Days to 50% maturity (days)	166.0±4.96^{a}	167.4±4.28^{a}	170.8±2.98^{a}	171.6±0.56^{a}
Number of pods plant^{-1}	40.9±4.01^{c}	192.4±1.77^{a}	185.9±0.38^{a}	78.6±1.96^{b}
Pod length plant^{-1} (cm)	7.17±0.14^{bc}	7.33±0.18^{ab}	7.21±0.16^{ab}	6.68±0.28c
Number of seeds pod^{-1}	9.00±0.23^{a}	5.1±0.18^{b}	4.4±0.22^{c}	4.7±0.21^{bc}
Seed yield plant^{-1}	179.2±0.73^{c}	946.4±0.18^{a}	892.8±0.63^{a}	409.8±4.11^{b}
100-seed weight (g)	16.23±0.64^{a}	14.41±1.14^{c}	13.86±0.04^{d}	14.92±0.80^{b}

Means followed with the same case letter along horizontal array indicate no significant effect (P > 0.05)

Phenotypic, genotypic environmental variances for the parental generations and M_1 were computed. It showed that the phenotypic variances for most traits were higher than the genotypic and environmental variances. Our result revealed that plant height plant^{-1}, number of leaves, leaf area, number of pod plant^{-1}, days to 50% flowering and maturity, seed yield plant^{-1} had high genotypic and phenotypic variances. Generally, the phenotypic variances were higher than genotypic variances, the variety and mutagenic concentration notwithstanding. M_1 generation progenies showed higher phenotypic and genotypic variances for number of leaves, number of flowers, days to maturity, number of pod plant^{-1}, 100-seed weight and seed yield plant^{-1} than the parental generation. On the other hand, parental generation recorded higher phenotypic and genotypic variations for plant height plant^{-1} and days to flowering when compared with the M_1 generation counterpart (Tables 6 & 7).

Comparing phenotypic, genotypic and environmental variances in parental and M_1 generations after APM treatment

In M_1 generation progenies, phenotypic and genotypic variances for number of leaves plant^{-1} were 766553.503 and 762399.28 (varietal effect) and 74193.3184 and 70039.0954 (treatment effect) while in parental generation,

Table 6. Phenotypic, genotypic and environmental variations in yield and yield-related traits obtained from parental lines that were treated with AMP

Morphological traits	Varietal effect			Treatment effect		
	Vg	Vp	Ve	Vg	Vp	Ve
Days to seedling emergence (days)	-0.3463	1.0717	1.418	-0.2205	1.1975	2206.1975
Percentage seed germination (%)	69.683	72.985	3.302	54.683	57.985	3.302
Plant height plant^{-1} (cm)	6473.310	6526.491	53.181	636.659	689.84	53.181
Number of leaves plant^{-1}	1015.765	1025.235	9.47	170.223	179.693	9.47
Number of branches plant^{-1}	34.90	35.577	0.677	1.797	2.474	0.677
Leaf area plant^{-1} (cm^2)	1042.05	1051.178	9.128	16.11	25.238	9.128
Internode length plant^{-1} (cm)	5.0678	6.157	1.0892	0.693	1.782	1.089
Petiole length plant^{-1} (cm)	2.359	2.463	0.104	0.241	0.345	0.104
Days to 50% flowering (days)	1137.074	1148.904	11.83	92.89	104.72	11.83
Number of flowers plant^{-1}	132.34	203.74	71.4	170.13	187.526	17.396
Days to 50% maturity (days)	323.765	329.205	5.44	49.078	54.518	5.44
Number of pod plant^{-1}	20.349	30.078	9.729	87.213	96.942	9.729
Pod length (cm)	0.350	0.849	0.499	0.255	0.755	0.5
Number of seeds pod^{-1}	-0.0468	0.6203	0.6671	0.009925	0.6769	0.666975
Seed yield plant^{-1}	2700.441	3073.805	373.364	2135.334	2508.698	373.364
100-seed weight (g)	3.736	5.657	1.921	0.618	2.539	1.921

Vp = phenotypic variance; Vg = genotypic variance, Ve = environmental variance

Table 7. Phenotypic, genotypic and environmental variations in yield and yield-related traits in M_1 generation of pigeon pea

Morphological traits	Varietal effect			Treatment effect		
	Vg	Vp	Ve	Vg	Vp	Ve
Percentage seed germination (%)	559	654	95	175	270	95
Days to seedling emergence (days)	-0.094	0.625	0.715	0.552	3.208	2.656
Plant height plant^{-1} (cm)	244.15	254.289	10.139	420.1795	433.009	12.8295
Number of leaves plant^{-1}	762399.28	766553.503	4154.223	70039.0954	74193.3184	4154.223
Number of branches plant^{-1}	-1.722	14.99	16.712	3.598	20.308	16.71
Internode length plant^{-1} (cm)	0.5699	2.0109	1.441	1.75	3.191	1.441
Petiole length plant^{-1} (cm)	-0.2978	1.3872	1.685	3.1804	4.8654	1.685
Days to 50% flowering (days)	1096.088	1106.266	10.178	6.713	16.891	10.178
Number of flowers plant^{-1}	1117.67	1854.545	736.875	20185.52	20922.395	736.875
Days to 50% maturity (days)	507.23	547.09	39.86	13.49	53.35	39.86
Pod length plant^{-1} (cm)	0.79	0.93	0.14	0.15	0.29	0.14
Number of seeds pod^{-1}	0.06	0.41	0.35	0.44	0.79	0.35
Number of pods plant^{-1}	7759.67	7900.08	140.41	11386.08	11526.78	140.7
Seed yield plant^{-1}	229866.83	235852.78	5985.95	33.67	125.65	91.98
100-seed weight (g)	43.24	43.71	0.47	4.85	5.31	0.46

Vp = phenotypic variance; Vg = genotypic variance, Ve = environmental variance the variances were 1025.235 and 1015.765 (varietal effect) and 179.693 and 170.223 (treatment effect). For seed yield, the variances were 235852.78; 229866.83 (varietal effect) and 125.65; 33.67 (treatment effect) in M_1 generation while parental generation had 3073.805; 2700.441 (varietal effect) with higher variances in treatment effect (2508.698; 2135.334). In all the traits, the environmental variances were the lowest.

Discussion

The essence of mutation breeding is to produce superior genotypes. However, it is very possible that mutation could cause enhancement in morphological traits and seed yield in the parental generation,but transfer of these modified genes into subsequent generation may be futile. It thus suggests that trait stability is fundamental in successful mutagenesis. It should be understood that it is at the point of trait stability that superior lines will be selected for mass production and possible commercialization. An interesting scenario played out in this current report. Results from the parental generation showed trait improvement as regards plant height, number of flowers, number of pods and seed yield though days to 50% flowering and maturity increased when seeds were exposed to 4 ppm APM. Udensi et al. (2011c) reported polyploidy inducing capacity of APM on pigeon pea varieties. This notwithstanding, the type of polyploidy induced is very cardinal inasmuch as it will determine the separation pattern of the chromatids to the poles. Udensi & Ntui (2013) observed that when the induced polyploidy is a mixoploid, it could either be a diploid+triploid (2n+3n) or diploid+tetraploid (2n+4n) and if tilts towards the former, there is every likelihood of aborted process as affirmed by Meng & Finn (2002) that fertility is often poor if the hybrid is triploid, pentaploid,or an aneuploid with a chromosome number less than hexaploid. This is a confirmation of the position of Udensi et al. (2011c) on induction of possible tetraploid at this concentration. This might be the underlying factor responsible for the excellent morphological performances in our current report.

It could also be observed that there was a reduction of days it took white "Fiofio" variety to flower (~160 days) as against brown "Fiofio" variety (183 days) while days to 50% maturity was increased in white (~198 days) but reduced in brown variety (~185 days). This discrepancy did not affect the yield in the parental generation. Obviously, it would be wise to assert that plants that first reached anthesis should also be the first to mature. However, most times it does not present itself as such. Though the mechanism underlying the phenomenon is presently unknown, it will be right to assume that the variety to reach anthesis first does not necessarily imply that the variety will mature first (Udensi et al., 2012a, b). When the M_1 seeds were planted for releasing the genetic variability through segregation and also to assess if the traits seemingly improved in the parental generation were stabilized, it was observe that number of leaves, number of flowers, number of pods, pod length, number of seeds pod^{-1} and seed yield were enhanced drastically, which could indicate traits stability in the M_1 generation. When the parental generation was assessed, pod length and number of seeds pod^{-1} were not significantly improved, APM concentration notwithstanding. However, in the M_1 generation, these two traits were significantly improved at 4 ppm APM. This is informative as the mutagenic effect on these traits manifested on the M_1 generation. The yield improvement observed in the M_1 generation might be linked to the integral contributions of other yield related traits such as number of leaves, leaf area, number of flowers, which had influenced pod production that culminates to increase seed yield (Udensi et al., 2011a, 2012a, b). Udensi & Ntui (2013) reported colchicine induced mutation leading to production of tetraploid (4n) and mixoploid (2n+4n) in pigeon pea while Brisibe et al. (2011) reported same for oryzalin in Egusi melon. It is most probabl that though Udensi et al. (2011c) reported polyploidy induction at other concentration of APM other than 4 ppm, the polyploidy may have been triploid (3n) or diploid-triploid (2n+3n), which might not be favourable for fertility This might answer why they may not have performed well morphologically.

Expectedly, increase in leaf number with broader leaf area should increase plant height, producing more branches, leading to increase seed yield (Udensi et al., 2011a, 2012a, b,). It should be realized that production of more flowers is not a prerequisite for higher pod production. On one hand, some flowers produced may not develop into pods and on the other, since yield is polygenic in inheritance; productivity is usually linked covalently to other factors. It therefore suggest that selecting superior pigeon pea genotypes, a plant breeder should select genotypes with good biomass yield, increased number of branches, flowers, pod length, etc., and not only on yield.

There were high and wide phenotypic and genotypic variances in some morphological traits, especially those traits that are seemingly concerned with yield in this present study. This was also reported by Tyagi et al. (2000); Sarsamkar et al. (2008); Idahosa et al. (2010); Udensi et al. (2011a; 2012a). Genotypic and phenotypic variances for most traits were higher in the M_1 generations than the parental generation, which is in conformity with earlier reports of Rohman et al. (2003) and Shamin (2012) in mungbean; Makeen et al. (2007) in *Vigna radiate*; Farshader & Farshader (2008) and Wani et al. (2012) in *Cicer arientinum*; Geeta & Manish (2011) in soybean. Generally, PV was higher than GV in almost all the traits, which according to Udensi et al. (2011a; 2012a) is an indication that pigeon pea yield improvement will be done majorly through either varietal and/or treatment-based phenotypic selection of traits. Selection of important agronomic traits in pigeon pea and indeed other crops revolves on the extent of genetic variability and obviously the degree to which the traits are inherited (Udensi et al., 2012a). Undoubtedly, improving traits with very small genetic control relative to environmental influences will be difficult (Ragsdale & Smith, 2003).

Further improvement and subsequent commercialization of any crop variety is a function of the extent of stability of traits after series of generations of mutation breeding (trials of mutant generations). It is therefore important to compare the performance of the parental and M_1 generations as to ascertain possible introgression of genes for releasing the genetic blueprint through segregation in the subsequent generations and their stability. From our current report, it is very clear that since plant height, number of leaves, number of flowers, number of pods, number of seeds pod^{-1} and seed yield recorded high variances, it does suggest that additive genes were strong and traits might be tilting stability in M_1 generation, which will be seen as the segregate in the M_2 and other generations.

Since seed yield is of utmost importance in pigeon pea breeding, it does therefore suggest that all breeding technique should be geared towards improving yield if food security will be a reality in the Sub-Saharan African countries. In the parental generation, APM treatment did not significantly affect yield but in the M_1 generation, white "Fiofio" variety produced more flowers plant^{-1} (531.4±1.53), pod plan^{t-1} (267.8±1.93) and seed yield plant^{-1} (1344.0±2.48), especially for the 4 ppm soaked seeds. Other trait such as number of leaves plant^{-1}, number of branches plant^{-1}, pod length competed favourably with plants raised from brown Fiofio at the same concentration. This was also reported by Udensi et al. (2012d) when they irradiated pigeon pea seeds with gamma rays. Additionally, though plants raised from seeds soaked in 4 ppm APM produced 398 seeds in parental generation, the production increased exponentially in the M_1 generation to 947 seeds (from means separation tables 5 & 8). Undoubtedly, treating pigeon pea with seeds with APM comparing the parental and M_1 generations giving the phenotypic and genotypic variances has induced positive significant variability, which are worth monitoring (Mahandjiev et al., 2001; Ciftci et al., 2006; Boureima et al., 2009).

Conclusions

Explicitly, there were obvious positive significant effects of APM treatment on pigeon pea, especially the white fiofio variety at 4 ppm. M_1 generation progenies performed better than their parental counterparts in yield traits, including seed yield, which could indicate success in mutagenesis at least in M_1. The treatment also caused high phenotypic and genotypic variances; especially in the M_1 generation. The implication is that M_1 seeds can be advanced to M_2 generation where genetic blueprint will be released through segregation.

Table 8. Mean separation of yield and yield related morphological traits of parental lines treated with AMP in respect to the treatments

Morphological traits	Concentration of amiprophos methyl used (ppm)			
	Control	4	6	8
Days to seedling emergence (days)	4.38±0.26a	4.38±0.83a	4.00±0.01a	4.63±0.26a
Percentage seed germination (%)	99.98±1.01a	95.25±2.62b	96.00±2.76b	87.5±0.13c
Plant height plant^{-1} (cm)	242.85±2.21d	261.55±2.34^{bc}	257.05±0.67c	263.35±0.34a
Numbers of leaves plant^{-1}	310.75±1.14a	289.38±1.86d	294.13±2.44c	301.13±0.91a
Number of branches plant^{-1}	15.5±0.5ab	14.50±1.43c	14.38±2.45c	16.5±0.80a
Leaf area plant^{-1} (cm^2)	69.99±0.56a	71.7±1.55a	69.29±3.22a	72.67±0.45a
Internode length plant^{-1} (cm)	6.63±0.61a	5.31±0.48b^{c}	5.10±0.21bc	4.8±0.12c
Petiole length plant^{-1} (cm)	3.73±0.30bc^{c}	3.75±0.33bc	3.70±0.13c	4.25±0.25a
Days to 50% flowering (days)	170.38±3.12b	179.38±2.21a	162.5±2.11c	172.0±0.99ab
Number of flowers plant^{-1}	108.63±3.21bc	117.7±1.79b	120.125±0.21a	101.0±0.99c
Days to 50% maturity (days)	192.88±3.98b	197.38±3.01a	186.25±1.47c	188±1.81c
Number of pods plant^{-1}	73.5±2.04^{b}	79.63±0.45a	67.13±2.11c	64.75±2.01c
Pod length plant^{-1} (cm)	6.34±0.31^{a}	6.0±0.34a	5.86±0.16a	6.86±0.44a
Number of seed pod^{-1}	4.88±0.23^{a}	5.0±0.21a	4.88±0.35a	5.53±0.26a
Seed yield plant^{-1}	361.38±1.87^{b}	397.5±0.78a	318.75±3.221c	341.5±0.56bc
100-seed weight (g)	12.82±0.525^{a}	13.60±0.52a	11.95±0.39a	13.38±0.53a

Means followed with the same case letter along horizontal array indicate no significant effect (P > 0.05)

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