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Induction of Genetic Variability in Cowpea Variety *Videza* for Extra Earliness and High Seed Yield using Gamma Irradiation Mutagenesis

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ABSTRACT

Background: Cowpea yields are very low in the West African region due to the prevalence of drought periods. This work, therefore, aimed at developing cowpea genotypes that combine early maturity with high seed yield, through gamma irradiation mutagenesis.
Methods: A farmer preferred cowpea variety *Videza* was improved *via* gamma mutagenesis. A determined lethal dose 50% (LD_{50}) of 240 Gy was used to irradiate 2000 seeds of *Videza* by applying a Cobalt 60 source. Selected M_1 generation plants exhibiting early maturity and high seed yield were advanced to M_2 and further to M_3 using *Videza* as parental control.
Result: Compared to the control *Videza,* the number of days to 90% maturity significantly decreased in putative mutants in the M_2 (from 71 days in the control to between 50 and 66 days in mutants) and further decreased in the M_3 where mutants matured 10-22 days earlier than the control. Significant increment in 100-seed weight per plant occurred in the M_3 mutants. In the M_3, 100-seed weight increased from 15.28 g in *Videza* to 21.71 g, the highest in mutants. Twelve putative mutants were identified that combine early maturity with higher seed yield than the control.

Key words: Agronomic trait, Food security, Mutagenesis, Plant breeding, Putative mutants, Severe drought.

INTRODUCTION

Cowpea production, processing and marketing are mainly undertaken by smallholder farmers (Odendo *et al.* 2011). Across Sub Saharan Africa, cowpea production is limited by frequent droughts that affect, especially the flowering and pod filling stages. According to Agbicodo *et al.* (2009), cowpea crop exposed to moisture stress at early flowering and pod filling stage results in yield losses of up to 80%. The cultivation of early maturing varieties is usually planned such that the crop could escape the drought periods and pests infestations (Selvan *et al.* 2021; Ehlers and Hall, 1997).

Cowpea genetic variability is quite narrow due to the self-pollinating nature (Asare *et al.* 2010). Besides, in Ghana, early maturing widely grown varieties such as *Sanzi* and *Asuntem* developed through conventional breeding, lack the following consumer preferred traits: cream seed coat colour, large grain size and early maturity as well as pests and disease resistance (Owusu *et al.* 2018).

To circumvent the effects of severe droughts, farmers prefer early maturing crops (Fatokun *et al.* 2012). In most self-pollinated plants where genetic variability is inadequate, induced mutation using irradiation has been an effective tool for creating significant genetic variability. Mutagenesis is faster and more effective compared to conventional breeding (Subba *et al.* 2022). Induced mutation has been successfully applied to create variability and improve many useful traits in cowpea and other crops (Ahloowalia *et al.* 2004). The aim of this study was to develop early maturing and high seed yielding cowpea varieties using gamma irradiation.

Phenotypes created by induced mutation are generally not readily observable in the M_1 generation due to resulting heterogeneous or chimeric plants (Jankowicz-Cieslak *et al.* 2017). It is, therefore, useful to advance M_1 plants to the M_2 generation and subsequent generations. Jankowicz-Cieslak *et al.* (2017) recommended the start of phenotypic screening for observable traits from the M_2.

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MATERIALS AND METHODS

Plant materials and irradiation dose used

A farmer-preferred cowpea variety, *Videza* was used. *Videza*, has a semi-erect growth habit, ovoid seed shape and smooth seed coat texture. The variety matures in 68-77 days and produces a maximum seed yield of 2261.3 kg/ha in the major rainy season (April to July) and 1375.0 kg/ha during the minor season (September to November) (Agyeman *et al.* 2015). The research was conducted at the Biotechnology and Nuclear Agriculture Research Institute (BNARI), Ghana, between March 2018 and April 2018. *Videza* was first planted at the BNARI and harvested seeds were multiplied for two consecutive generations to develop a single seed descend population of approximately 8000 seeds for irradiation. A radio-sensitivity test was carried out following established protocols (Mba *et al.* 2010) to determine a dose rate of 240 Gy for mass irradiation. Thirty seeds per dose were gamma irradiated in March 2019 using a Cobalt 60 source.

Treatment of seeds and raising M_1, M_2 and M_3 populations

About 2000 seeds were acutely irradiated at 240 Gy. Non-irradiated materials served as control. The M_1 seeds were planted under normal growing conditions. The M_1 field comprised 10 rows of irradiated seeds separated by a spacing of 1.50 m from three rows of control fields. Induced M_1 plants with early maturity and large seed size were selected and the M_2 seeds harvested individually. All M_2 seeds were used to establish the M_2 mutant population as described for the M_1 population. Data on the M_2 generation were recorded from ten plants in each progeny row. At maturity, tagged induced M_2 lines from each progeny line with early maturity and high seed yield were selected and M_3 seeds harvested individually. The M_3 seeds were sown as single-plant progenies to establish the M_3 generation. Induced M_3 plants from each progeny line with early maturing and high seed size were harvested separately.

RESULTS AND DISCUSSION

Number of days (ND) to 50% flowering at M_2 and M_3 generations

ND to 50% flowering at M_2 and M_3 are presented in Fig 1. In the M_2, ND to 50% flowering ranged from 32 days to 46 days among the M_2 lines. The earliest to attain 50% flowering was in putative mutant P3N01 while the latest was in P10N29. The control plant flowered at 47 DAP. In the M_3, ND to 50% flowering ranged from 30 days to 36 days among the M_3 lines. Mutants P1N02#4, P3N01#5, P4N03#2 and P4N14#7 flowered on day 30 after planting. The latest to attain 50% flowering were P2N09#16 and P6N10#19. The control plants in the M_3 attained 50% flowering at 46 DAP.

ND to 50% flowering reduced among the selected mutants compared to the control in the M_2. Some of the mutants flowered 10-15 days earlier than the control *Videza.* The mutant P3N01 was first to flower at 32 DAP while the control line flowered at 47 DAP. In the M_3, further reduction in days to 50% flowering (ranged from 30 days to 36 days) was observed in M_3 mutants compared to the M_2. A similar result was reported by Horn (2016) in cowpea and Shamsun *et al.* (2018) in chickpea. The reduction in flowering time offers the possibility for selecting early maturing mutants.

Number of days (ND) to 90% maturity at M_2 and M_3 generations

Fig 2 displays ND to 90% in the M_2 and M_3. Days to 90% maturity ranged from 50 to 66 days among the putative mutants in the M_2. The earliest maturing mutants were P1N02, P3N01 and P4N03 while the latest was P10N30. The control plant in M_2 attained 90% maturity at 71 DAP. In the M_3, ND to 90% maturity ranged from 48 to 60 days. The earliest to attain 90% maturity was mutant P1N02#1, while the latest were P2N09#16 and P6N10#19. The control attained 90% maturity at 70 DAP.

It was observed that some of the mutants at the M_3 matured 10-22 days earlier than the control. Reductions in days to 90% maturity due to gamma radiation treatment have been reported in many crops including cowpea (Horn, 2016) and chickpea (Shamsun *et al.* 2018). Early maturity is desirable for cowpea cultivation in most growing regions due to the significant loss in yield particularly when the flowering and pod filling stages coincide with long drought periods. Early maturity enables the crop to escape drought during the reproductive phase (Bowles *et al.* 2021). The reduction in days to 90% maturity and increased variability in yield characters proved that genetic variability induced by gamma irradiation is in the desired direction and offers an effective means for selecting early maturing and high seed yield mutants. Horn (2016) and Otusanya *et al.* 2022 recorded significant differences in ND to 90% maturity, 100-seed weight, seed yield per plant, number of pods per plant in M_2.

Number of pods per plant (NPP) at M_2 and M_3 generations

NPP at the M_2 and M_3 are shown in Fig 3. NPP ranged from 10.00 to 45.00 among the putative mutants in M_2. The highest NPP was observed in P1N05 while the lowest was recorded in P8N15. By comparison, the control lines produced a mean number of 28.90 pods per plant. In the M_3, NPP ranged from 26.00 to 47.00 among selected mutants. The mutant P4N03#2 had the highest NPP (47.00) while the lowest (26.00) was observed in P1N08#13. The control line planted alongside the M_3 mutants produced a mean of 30.00 pods per plant.

NPP increased in some of the selected mutants compared to the control in the M_2. NPP increased further in the M_3. Increase in NPP after mutagenic treatment has also been reported in cowpea (Horn, 2016) and chickpea (Wani, 2011). Increase in NPP has been attributable to an increase in number of flowers in cowpea (Dingha *et al.* 2021). This trait plays a vital role in increased seed yield per plant in cowpea.

Number of seeds per pod (NSP) at M_2 and M_3 generations

NSP obtained in selected putative mutants at the M_2 and M_3 are presented in Fig 4. In the M_2, the mean NSP varied from 10.25 to 22.50 among the putative mutants. The mutant P5N11 produced the highest NSP while P9N12 and P8N24 produced the lowest NSP. The control lines produced a mean of 12.25 of seeds per pod. NSP varied from 12.50 to 23.00 among selected mutants in M_3. The highest NSP was observed in P5N07#8 while P1N06#9 had the lowest number of seeds per pod. The control lines produced a mean of 13.00 seeds per pod.

NSP increased in some of the selected mutants compared to the control. Data on the mean NSP clearly showed that the mutants with the highest NSP also produced

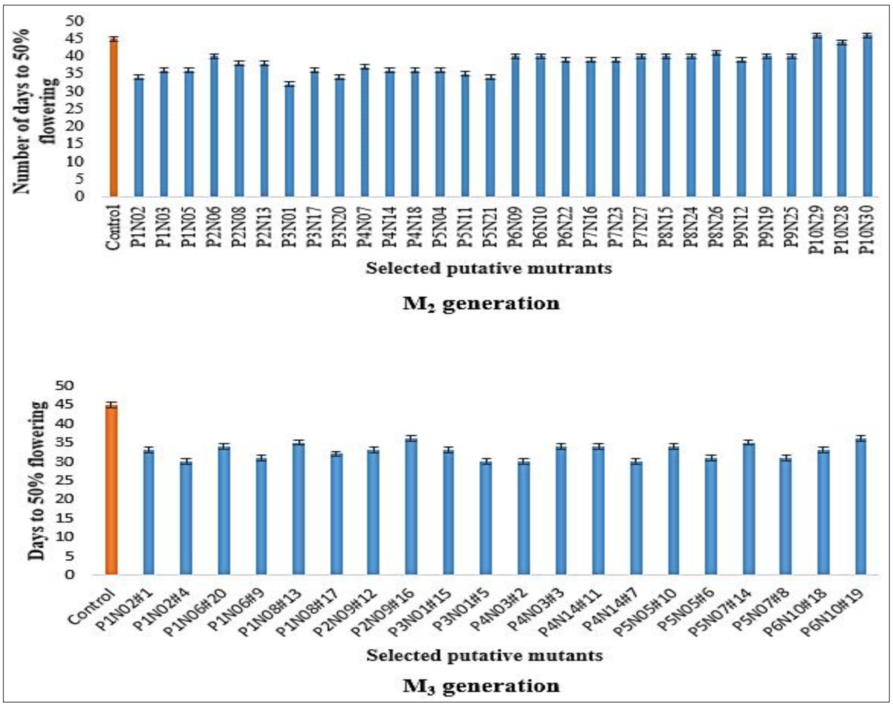

Fig 1: Number of days to 50% flowering at M_2 and M_3 generations.

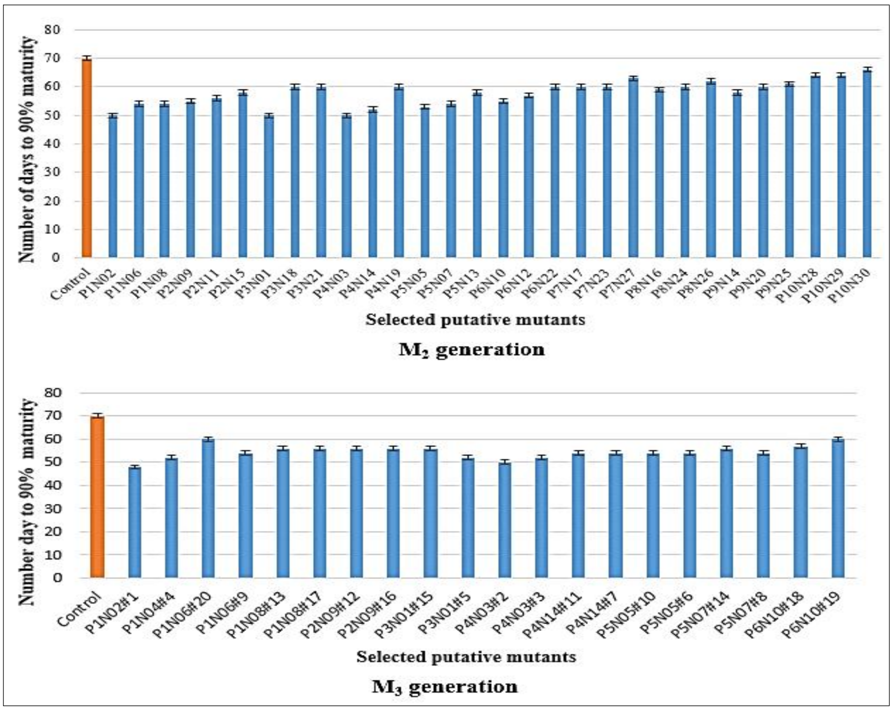

Fig 2: Number of days to 90% maturity at M2 and M_3 generations.

the longest pod lengths, signifying a close relationship between these two traits. Even though the mean pod length increased slightly in the M_3 plants, no substantial changes in the mean NSP were observed. Similar observations were reported in soybean (Justin *et al.* 2012) and lentil (Laskar and Khan, 2017). Similarly, Khan and Wani (2006) did not observe significant differences in NSP in the M_2 and subsequent generations after gamma radiation treatments. The increase in NSP translates into high seed yielding putative mutants.

Hundred-seed weight per plant (HSWP) at M_2 and M_3 generations

HSWP among the selected putative mutants at the M_2 and M_3 are presented in Fig 5. In the M_2, HSWP varied from 13.85 g to 22.80 g. The mutant P5N21 had the highest

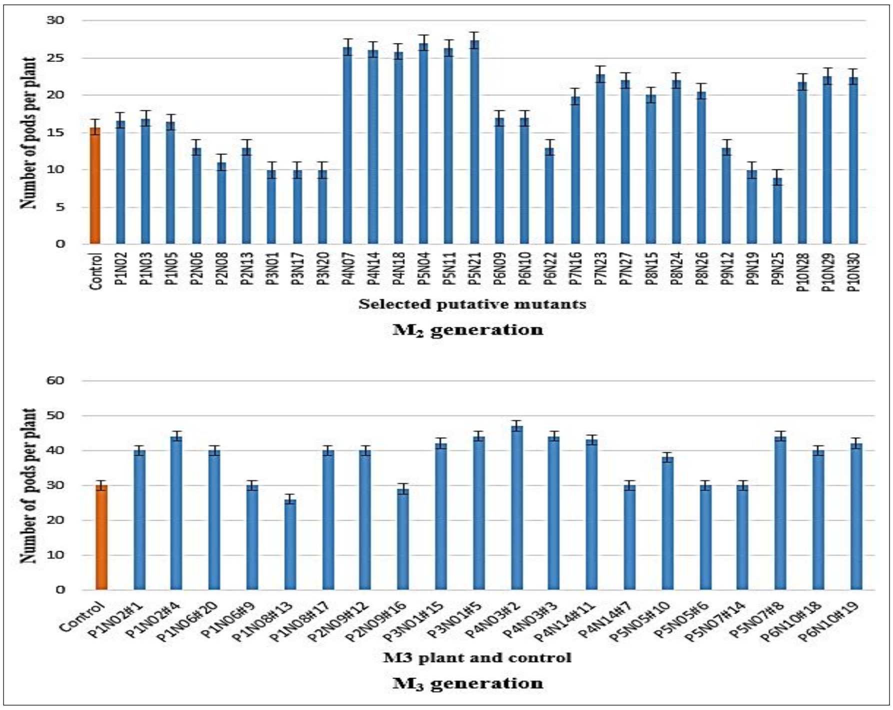

Fig 3: Number of pods per plant at M_2 and M_3 generations.

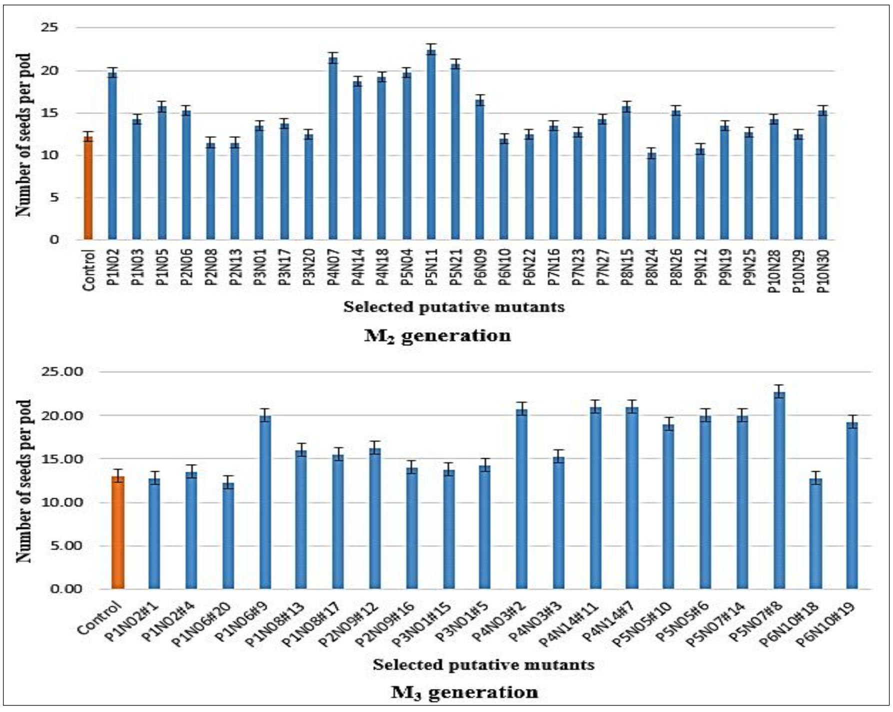

Fig 4: Number of seeds per pod at M_2 and M_3 generations.

HSWP (22.80 g) while the lowest HSWP was observed in P8N24 (13.85 g). The control line gave a mean of 16.95 g for HSWP. HSWP ranged from 12.69 g to 21.71 g in the M_3. The highest 100-seed weight was observed in P6N10#19 and the lowest was recorded in P4N14#11. The control line produced a mean of 15.28 g for HSWP.

Hundred seed weight is considered as one of the most reliable yield parameters for measuring seed yield in grain legumes. An increase in 100-seed weight was recorded in some of the selected mutants compared to the control in the M_2 (from 16.95 g in the control to 22.80 g in mutant lines). A similar trend was observed in the M_3 as was

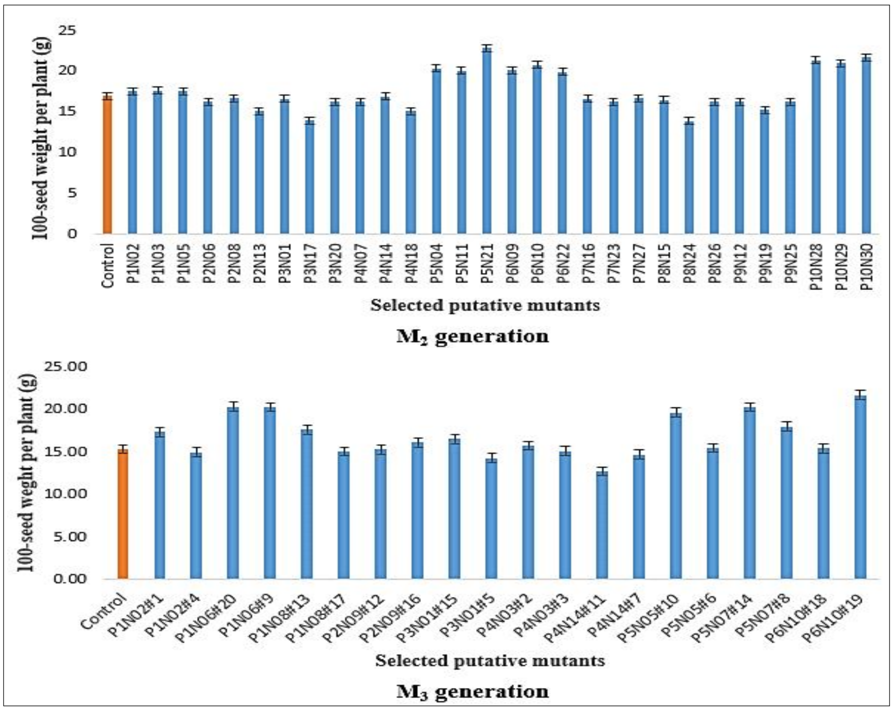

Fig 5: 100-seed weight per plant at M_2 and M_3 generations.

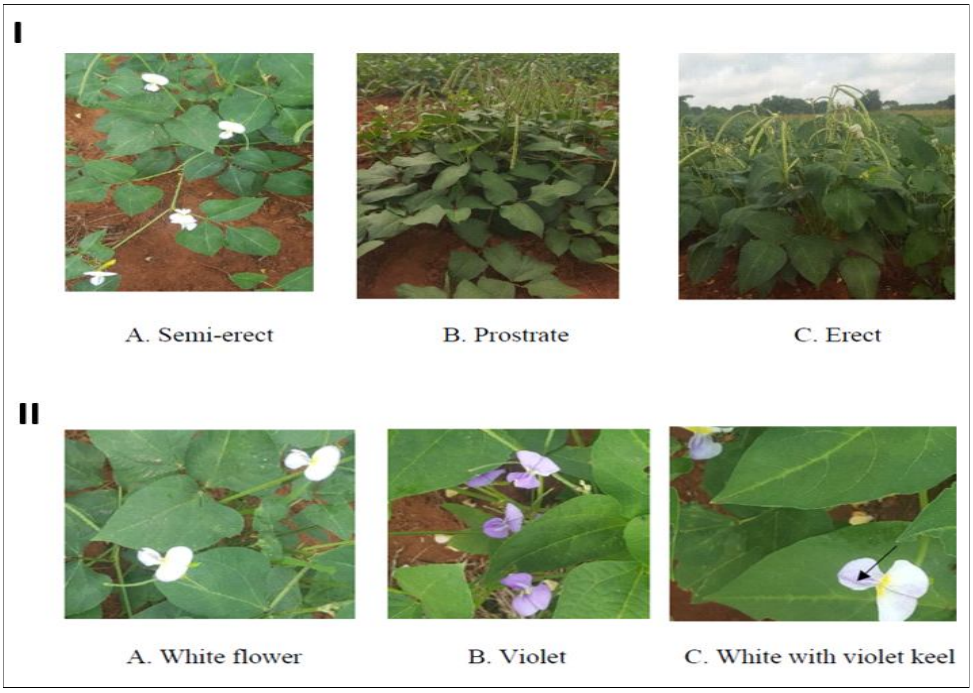

Fig 6: I. Growth habit variations at M_3 generation. Control (A) and M_3 plants (B and C); II. Flower colour variations at M_3 generation. Control (A) and M_3 plants (B and C).

recorded in the M_2 for HSWP. 100-seed weight increased in some of the selected mutants at M_3 generation (from 15.28 g in the control to 21.71 g in mutant lines). Horn (2016) also reported significant improvement in HSWP in cowpea mutants after gamma irradiation. Similarity, Yuliasti and Reflinur (2018) reported an increase in 100-seed weight in soybean mutants. The observed increase in HSWP indicates the practicality of achieving large-seeded putative mutants via further selection.

Putative mutant lines exhibiting earliness and high seed yield at the M_3 generation

Rankings of putative mutants exhibiting combined earliness and high seed yield at the M_3 are presented in Table 1. Putative mutants P1N06#9, P1N02#, P1N08#13, P1N08#17, P1N06#20, P1N08#17 P2N09#12, P4N03#2, P5N05#10 and P5N07#8 were observed to exhibit earliness as well as high seed yield. The lower the sum of ranking, the higher the position of the ranked genotypes.

Fig 7: I. Seed eye colour deviations at M3 generation. Control (A) and M_3 plants (B and C); II. Seed coat colour variations at M_3 generation. Control (A) and M_3 plants (B, C, D and E).

Table 1: Putative mutant lines exhibiting earliness and high seed yield at M_3 generation.

Control/ Putative mutant	Earliness (Days to 90% maturity)		Seed yield per plant		Summary of rankings	
	Value recorded	Ranking	Value recorded	Ranking	Sum	Final ranking
Control	70	8	48.65	11	19	13
P1N02#1	48	1	46.60	13	14	10*
P1N02#4	52	3	20.62	20	23	19
P1N06#20	60	7	65.36	6	13	9*
P1N06#9	54	4	97.38	1	5	1*
P1N08#13	56	5	81.24	3	8	3*
P1N08#17	56	5	56.23	9	14	10*
P2N09#12	56	5	73.94	4	9	4*
P2N09#16	56	5	31.38	17	22	18
P3N01#15	56	5	38.85	14	19	13
P3N01#5	52	3	27.28	18	21	16
P4N03#2	50	2	51.36	10	12	5*
P4N03#3	52	3	19.12	21	24	21
P4N14#11	54	4	26.18	19	23	20
P4N14#7	54	4	58.05	8	12	5*
P5N05#10	54	4	95.97	2	6	2*
P5N05#6	54	4	31.41	16	20	15
P5N07#14	56	5	61.42	7	12	5*
P5N07#8	54	4	46.86	12	16	12*
P6N10#18	57	6	35.01	15	21	17
P6N10#19	60	7	70.83	5	12	5*

* indicates putative mutants exhibiting both earliness and high seed yield traits.

Growth habit and flower colour variation

Fig 6 I and II show the growth habit and flower colour variations at the M_3. In the M_3, some of the induced lines changed from the semi-erect parental control growth habit to prostrate or erect growth habit in mutants (Fig 6 I). Flower colour variations at the M_3 are displayed in Fig 6 II. *Videza* produces white flowers. However, at the M_3, some of the mutagenized plants produced violet and white with violet flower colours.

Seed eye and coat colour variation

Seed eye and coat colour variations observed at the M_3 are shown in Fig 7 I and II. *Videza* has black eye. Seeds with brown or brown splash eye developed at the M_3 in the induced lines (Fig 7 I). Seed coat colour variations at the M_3 generation are displayed in Fig 7 II. The seed coat of Videza is white. However, brown or grey speckled, brown holstein or grey Holstein coats were observed in the seeds of M_3 lines.

Seed shape, seed texture and pod length variation

Shown in Fig 8 I and II are the seed shape and testa texture variations at the M_3 generation. *Videza* characteristically produces kidney shaped seeds. However, at M_3, globose and Rhomboid shaped beans were obtained (Fig 8 I). Seed testa texture variations at the M_3 generation are shown in Fig 8 II. *Videza* produced smooth to rough testa texture

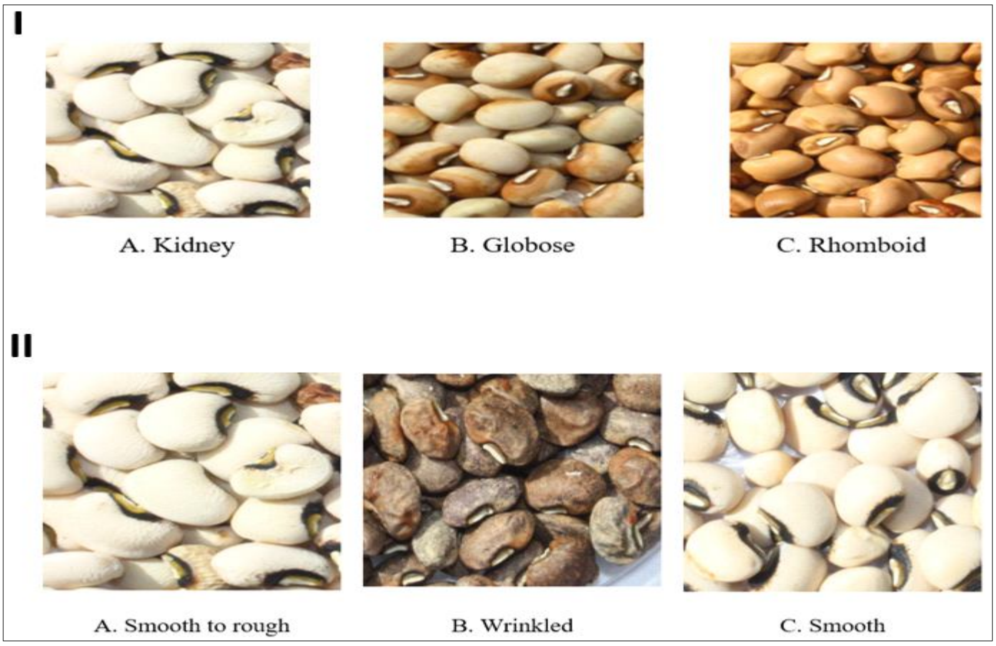

Fig 8: I. Seed shape variations at M_3 generation. Control (A) and M_3 plants (B and C); II. Seed testa texture variations at M3 generation. Control (A) and M_3 plants (B and C).

Fig 9: Pod length variations at M_3 generation.

compared to the texture of some of the induced lines with smooth, rough to wrinkled or wrinkled seed. Pod length variations at the M_3 compared to *Videza* are shown in Fig 9. In the M_3, some of the putative mutants had pod lengths significantly longer than the control. Edematie *et al.* 2021 explained that it is feasible to improve seed yield in cowpea *via* screening for long pods.

CONCLUSION

Twenty putative mutants exhibited significantly early maturity taking between 50 and 60 days to attain 90% pod maturity compared to parental control *Videza* lines which took 70 days. Ten mutants produced significantly higher seed yield per plant ranging 51.36 g - 97.38 g than the Videza lines with a mean of 48.65 g. Twelve putative mutant lines were ranked to outperform Videza with respect to earliness and seed yield per plant.

Conflict of interest: None.

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