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Chapter 4

Potential of Improving Agronomic Attributes in Tropical Legumes Using Two Mutation Breeding Techniques in Southern Africa

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Additional information is available at the end of the chapter

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Abstract

Tropical legumes such as cowpea (*Vigna unguiculata*) and tepary bean (*Phaseolus acutifolius*) are important in traditional smallholder cropping systems, particularly in sub-Saharan Africa. Both legumes are adapted to harsh environments including extreme temperatures, drought and poor soil fertility. They provide affordable sources of protein for human consumption and are valuable for income generation. These crops contribute significantly to soil fertility improvement through biological nitrogen fixation. In many parts of Africa, the productivity of these legumes is generally low partly because farmers grow unimproved varieties that are often produced for subsistence purposes on poor soils in mixed cropping systems with limited production inputs. Therefore, this research was designed to evaluate the potential of two distinct mutation breeding approaches in creating useful genetic variation in the two legumes in order to improve the agronomic attributes of both crops. The variation was determined by measuring a range of agronomic traits at both the seedling and adult plant stages. The results showed significant genetic variation among cowpea mutants that were induced with various doses of gamma radiation as well as among tepary bean mutants that were induced with a chemical mutagenic agent, ethyl methanesulphonate (EMS). The optimum doses at LD_{50} for two cowpea genotypes (Nakare and Shindimba) were ≤200 Gy while the third genotype (Bira) tolerated a dose three-fold higher. In the EMS mutagenesis of tepary bean, the estimated LD_{50} was ≤2.4% EMS (v/v). In both approaches, percent seed germination decreased with increased dose and the coefficients of determination for the linear functions were high (>75%), suggesting that there were notable associations between the reduction in seed germination and the concentration of the mutagen. At the adult plant stage, tepary bean showed that the mutant generation significantly ($P < 0.05$) influenced positively the important agronomic traits such as shoot dry weight, number of pods per branch and seed size. Dose effects were also significant for seed size. The field trials conducted in Zimbabwe showed >10.0% increase in both seed size and grain yield potential of some mutant cowpea genotypes compared with the standard check. These findings provide

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reference doses for large-scale gamma irradiation of cowpea as well as chemical mutagenesis for tepary bean. In addition, the germplasm produced from these approaches has the potential for selection in a range of agro-ecological conditions across the region, thus creating alternative cropping systems for the smallholder growers.

Keywords: cowpea, genotype, legumes, mutagenesis, tepary bean

1. Introduction

Tropical legumes such as cowpea (*Vigna unguiculata*) and tepary bean (*Phaseolus acutifolius*) are important in traditional smallholder cropping systems, particularly in sub-Saharan Africa. Both legumes are adapted to harsh environments including extreme temperatures, drought and poor soil fertility [1–3]. Cowpea was domesticated in Southern Africa where its wild relatives are found [4] and it is cultivated widely in many countries in the region including Namibia, South Africa and Zimbabwe. In contrast, tepary bean is indigenous to the south-western parts of the United States and Mexico [5], but spread to many African countries including Botswana, Kenya, Malawi, South Africa and Zimbabwe where smallholder farmers use unimproved landraces of the crop. The grain of both cowpea and tepary bean provide affordable sources of protein for human consumption and are valuable for income generation, particularly in the smallholder cropping systems in southern Africa. In addition, the legumes contribute significantly to soil fertility improvement through biological nitrogen fixation [6,7] and are often produced in intercrops with maize (*Zea mays*), sorghum (*Sorghum bicolor*) and millets (*Pennisetum* spp.) that are popular in the region.

Despite these important uses, the productivity of these legumes is generally low (<500.0 kg/ha), partly because farmers grow unimproved varieties which are often produced for subsistence purposes on poor soils in mixed cropping systems with limited production inputs. In addition, the genetic base of each of these legumes is narrow, particularly for exploiting important economic traits such as grain yield and tolerance to insect pests. However, mutation breeding has the potential to generate unique genetic variations in crops that can be exploited by plant breeders in the development of new cultivars [8]. The success of mutation breeding has been reported widely in legumes [9–13], cereals [14,15] and several other crops such as sunflower [16], cassava [17] and oilseed rape [18]. In mutation breeding, artificial mutagenesis is induced on crop germplasm using various types of mutagenic agents such as gamma rays or ethyl methanesulphonate (EMS) followed by selection of useful traits from the resulting mutants. The approach has the potential to produce desirable results faster than conventional plant breeding methods [19]. In this chapter, we present research work which was designed to evaluate the potential of two distinct mutation breeding approaches in creating useful genetic variation in cowpea and tepary bean in order to improve the agronomic attributes of both crops. The development of improved cultivars of these legumes using diverse germplasm from southern Africa will benefit breeding programs as well as growers and end-users in the region.

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2. Materials and methods

The study consisted of three components involving separate artificial mutagenesis of batches of cowpea seeds and tepary bean, followed by evaluation of seedlings under greenhouse or laboratory conditions. In addition, the agronomic field performance of both cowpea and tepary bean mutants was conducted. The field evaluation of cowpea mutant lines utilized distinct agro-ecological conditions in Zimbabwe (**Table 1**). The early generations of tepary bean mutants were evaluated at a representative location in the semi-arid region of northern South Africa.

Test location	Co-ordinates	Soil type	Annual rainfall (mm)	Summer temperatures (°C)
Thohoyandou (South Africa)	22°58'S; 30°26'E	Red well- drained, clay	500 to 920	22–40
Gwebi Variety Testing Centre (Zimbabwe)	17°41′S 30°32′E	Red clay	700–1000	17–30
Save Valley Research Station (Zimbabwe)	21°02′S 31°57′E	Alluvial	<450	15–37
Matopos Research Station (Zimbabwe)	20°24′S 28°28′E	Black clays	450–650	15–29

Table 1. The characteristics of the test locations used in the study.

2.1. Cowpea genetic material and its mutagenesis

In the first part of the study, batches of about 150 seeds (in three replications) of each of three cowpea genotypes (Nakare, Shindimba and Bira) were gamma-irradiated at the facility at the International Atomic Energy Agency (IAEA), Agriculture and Biotechnology Laboratory (Austria) using a range of irradiation doses (0, 100, 200, 300, 400, 500 and 600 Gy) [20] in order to determine the optimum irradiation level causing optimum mutation frequency and subsequently planted in a greenhouse. Similarly, the M_1 seed of one cowpea genotype (CBC-1, originating from Zimbabwe), which was gamma-irradiated at the same facility in Austria, was planted in the greenhouse initially in order to raise M_2 plants. Subsequent successive generations (from which several experimental lines were isolated) were raised at the Crop Breeding Institute (Harare, Zimbabwe).

2.1.1. Seedling evaluation and measurements

The mutagenized seeds (M_1) were planted in a greenhouse at the IAEA (Austria) in seedling trays with a medium consisting of peat, sand and vermiculate at a ratio of 2:1:1, respectively. The temperature and photoperiod in the greenhouse were maintained at 28.0°C and 12.0 h,

respectively. The soil moisture was maintained by watering twice per week. A completely randomized experimental design with three replications was used for the study. The percent germination (%G) was measured 7 days after planting, but the epicotyl and hypocotyl lengths were measured 14 days after planting. The measurements that were obtained for the %G were used for determining the LD_{50} for each genotype which was estimated with the aid of a linear regression model using a simple straight line equation $y = mx + c$ [where: y = the response variable (%G), x = the independent variable (irradiation dose); m = the slope and c = the constant].

2.1.2. Field evaluation and measurements

Fourteen M_6 cowpea mutant genotypes together with two standard checks were evaluated in the field at three distinct locations in Zimbabwe (**Table 1**). The seed of each genotype was planted in two-row field plots spaced at 0.45 m apart × 0.15 m intra-row spacing and arranged in a randomized complete block design replicated four times. Standard cowpea management practices were followed at Gwebi Variety Testing Centre (G.V.T.C.) and Matopos Research Station (M.R.S.). The field trial at Save Valley Research Station (S.V.R.S.) was conducted during the off-season period in winter under irrigation. The location experiences no frost in winter.

At each location, six agronomic traits were measured as follows:

(i) duration to 50.0% flowering (50%DF) (ii) duration to 95.0% maturity (95%DM) (iii) number of pods per plant (NPP) (iv) number of seeds per pod (NSP) (v) 100 seed weight (100-SW) and (vi) grain yield (GY).

2.2. Tepary bean genetic material and its mutagenesis

Three genotypes of tepary bean (GEN-1; GEN-4; GEN-6) that were obtained originally from growers in Sekhukhune District (Limpopo Province, South Africa) were used in the study. These genotypes were self-fertilized for five cycles in order to maximize homozygosity prior to chemical mutagenesis. The selfing was conducted in the greenhouse at the University of Venda (Thohoyandou, South Africa). The seed mutagenesis was conducted at the University of KwaZulu Natal (Pietermaritzburg, South Africa). A sample of healthy clean seeds (approximately 250) of each genotype was surface-sterilized by soaking in 70% ethanol for 1 min and rinsing three times followed by soaking in 30% sodium hypochlorite bleach solution (2% NaOCl) for 10 min before rinsing again three times in tap water. The seed was then soaked in distilled water for 12 h at room temperature. Each seed sample was partitioned into smaller batches (containing about 50 seeds each) and placed in specially designed sachets made of nylon mesh (measuring about 7.0 cm in width × 11.0 cm in length) [21]. The seed was transferred to aqueous solutions of varying doses (0.0, 0.5, 1.0, 1.5, 2.0 v/v) of EMS and incubated at room temperature for 1 h after which the treated seed was rinsed under running tap water for 2 h in order to remove the excess EMS and enable safe handling. A portion of the M_1 seed (75%) was used for evaluating the seedling traits while the remainder was planted in the greenhouse in order to raise the M_2 to M_4 seed for the subsequent field evaluation.

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2.2.1. Seedling evaluation and measurements

The mutagenized seed samples (M_1) were germinated in plastic jars (7.5 cm diameter × 8.0 cm height, lined with moist filter paper at the base) in the laboratory at room temperature. At the first initiation of the first trifoliate leaf, several seedling traits were measured including (i) percent seed germination (%G), (ii) number of secondary roots (NSR), (iii) primary root length (PRL) (mm), (iv) secondary root length (SRL) (mm) and (v) shoot height (mm) (SHT).

2.2.2. Field evaluation and measurements

The seed of each treatment combination (generation × genotype × dose) was planted separately (in plots spaced at 0.1 m within the row and 0.6 m between the rows) in the field under rain-fed conditions. A three-factorial arrangement laid out in a randomized complete block design was used in the study. At the reproductive stage or maturity, a range of agronomic attributes were measured including the (i) number of primary branches per plant (NPB) (ii) pod length (PL) (iii) shoot dry weight (SDW) (iv) 100 seed weight (100-SW).

3. Results and discussion

3.1. Seedling performance of cowpea mutants

A significant ($P < 0.01$) interaction occurred between cowpea genotypes and gamma radiation doses, suggesting that there were differential responses to the irradiation doses among the cultivars. The %G decreased drastically with increased irradiation dose in all the three cultivars. At 600.0 Gy, seed germination (47.9%) occurred only in cultivar 'Bira'. The highest LD_{50} (689.00 Gy) was observed for cultivar 'Bira' (**Table 2**), which suggested that this cultivar was the most resistant to gamma radiation.

Cowpea cultivar	Linear equation	LD_{50} (Gy)
Nakare	$y = -0.17x + 78.09$	165.24
Shindimba	$y = -0.16x + 81.79$	198.69
Bira	$y = -0.08x + 105.12$	689.00

Table 2. The LD_{50} of three cowpea cultivars that were irradiated with gamma rays in order to induce mutation.

Radiation doses >200.0 Gy significantly reduced the epicotyl and hypocotyl lengths in both 'Nakare' and 'Shindimba', but at 0.0 Gy the longest epicotyl (3.11 cm) and hypocotyl (6.71 cm) were observed in the cultivar 'Nakare' [20]. In general, both the epicotyl and the hypocotyl lengths decreased as the gamma irradiation doses increased. Between 400.0 and 600.0 Gy, the hypocotyl growth occurred only in cultivar 'Bira'.

The coefficient of determination (R^2) estimated from the linear regression equations for both traits were high (ranging from 76.0–93.0%), suggesting that there was a notable association

between the reduction of epicotyl and hypocotyl lengths due to increased radiation doses. These results showed genotypic variation in tolerance to gamma radiation with 'Bira' tolerating the heaviest doses of radiation. Therefore, from a crop improvement standpoint, it is critical to determine the optimum dose of irradiation for each candidate cowpea genotype prior to large-scale blanket mutagenesis with the gamma radiation approach. Ideally, the irradiation doses for generating useful mutants in crop improvement programs should be within ±5 units of the experimentally determined optimal dose [17]. Nonetheless, induced mutations are random events such that, in all probability, their reproducibility in each candidate genotype with the same mutagen is highly unlikely.

3.2. Agronomic field performance of cowpea mutants

During the advancement of the mutagenized generations, the cowpea mutants derived from 'CBC-1' using gamma radiation revealed two notable phenotypes namely earliness (**Figure 1**) and above canopy pod development (**Figure 2**). Early maturity is particularly important as a mechanism for escaping drought. There were significant ($P < 0.05$) differences only in the duration to 50.0% flowering (50%DF) and grain yield (GY) over the three consecutive cropping seasons (starting from 2012/2013) at G.V.T.C. in Zimbabwe. This indicated that, to all intents and purposes, the majority of the useful agronomic traits of the mutants were stable after the M_5 generation. At the location, the highest grain yield (0.87 t/ha) was attained by the mutant line 'CM/250/M6-6'. In all the selected elite lines, the desirable traits were induced by using a relatively low-range (150.0–250.0 Gy) dose of gamma radiation in the original parental line (CBC-1).

The results also showed that there was no significant variability for the duration to 95.0% maturity (95%DM) or the number of pods per plant (NPP) or the number of seeds per pod (NSP), suggesting that the selected elite lines were uniform with regard to these traits. However, the mean NPP (9.0) was considerably lower in comparison with observations from other studies [22]. In a similar study that evaluated cowpea M_1 genotypes, the NPP was reduced from 43 to 17 by exposure to 400.0 Gy [9]. However, the mean NSP was consistent with other findings [23].



Figure 1. Some cowpea mutant lines showed early maturity.

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Figure 2. Cowpea pods developed above the canopy in some mutant lines.

Mutant line	50%DF	95%DM	NPP	NSP	100-SW (g)	GY (t/ha)
CM/200/M6-2	60.00 a	95.66 a	21.33 a	15.33 a	14.33 abc	1.19 a
CBC-2	59.00 a	95.66 a	21.33 a	15.00 a	12.67 c	1.43 a
CM/250/M6-5	59.00 a	95.33 a	22.33 a	15.33 a	15.67 a	1.57 a
CM/250/M6-2	59.67 a	97.33 a	23.00 a	14.00 a	13.33 bc	1.21 a
CM/150/M6-1	60.67 a	97.33 a	28.00 a	15.00 a	15.67 a	1.72 a
CM/200/M6-3	60.67 a	95.33 a	22.33 a	14.00 a	15.67 a	1.15 a
CM/250/M6-1	59.67 a	95.67 a	21.67 a	13.67 a	15.00 ab	1.21 a
CM/250/M6-7	58.00 a	94.33 a	14.33 a	14.00 a	15.00 ab	1.28 a
CM/250/M6-4	60.67 a	94.33 a	22.67 a	15.00 a	15.00 ab	1.24 a
CBC-1	61.00 a	95.33 a	21.33 a	15.67 a	15.00 ab	1.29 a
CM/150/M6-3	60.33 a	96.33 a	18.33 a	14.33 a	14.33 abc	1.16 a
CM/200/M6-4	58.00 a	95.00 a	21.67 a	14.00 a	13.67 abc	1.38 a
CM/150/M6-2	59.33 a	94.33 a	18.33 a	13.33 a	15.33 ab	1.32 a
CM/200/M6-1	58.67 a	95.00 a	23.00 a	15.00 a	14.33 abc	1.41 a
CM/250/M6-6	61.00 a	94.67 a	18.00 a	13.33 a	14.33 abc	1.36 a
CM/250/M6-3	60.00 a	95.67 a	19.00 a	14.33 a	15.00 ab	1.21 a
Mean	59.73	95.46	21.02	14.46	14.64	1.32
C.V.	2.24	1.47	22.08	8.71	7.90	23.85
Significance	NS	NS	NS	NS	*	NS

Means followed by the same letter in each column are not significantly different ($P < 0.05$).
NS = not significant at the 5.0% probability level; * = not significant at the 5.0% probability level.
[50%DF = duration to 50% flowering; 95%DM = duration to 95% maturity; NPP = number of pods per plant; NSP = number of seeds per pod; 100-SW = 100 seed weight; GY = grain yield].

Table 3. Mean performance of cowpea M_6 experimental lines over two cropping seasons at the Matopos Research Station (Zimbabwe).

At M.R.S., the highest yield was attained by the experimental cultivar 'CM/150/M6-1' (**Table 3**). The average NPP (21.0) increased by more than two-fold (**Table 4**) while both the 50%DF and the 95%DM increased markedly in comparison with the other two locations, suggesting that the agro-ecological conditions were more favourable at this location than at G.V.T.C. for this sample of cowpea experimental lines. This trend was more apparent when the germplasm was evaluated during winter but under irrigation at the S.V.R.S. where all the genotypes attained >4.0 t/ha grain yield (**Figure 3**). The line 'CM/150/M6-1' achieved the highest (6.36 t/ha) which represented almost 20.0% grain yield advantage over the check cultivar. In addition, four genotypes achieved >10.0% higher grain yield over the check cultivar 'CBC-2' (**Figure 4**). These results demonstrated the potential of the newly developed genotypes to increase the productivity of cowpea significantly particularly under favourable conditions such as those at S.V.R.S.

Test location	50%DF (d)	95%DM (d)	NPP	NSP	100-SW (g)	GY (t/ha)
Gwebi Variety Testing Centre	53.50	78.78	9.00	11.78	13.91	0.75
Matopos Research Station	59.73	95.46	21.02	14.46	14.64	1.32
Save Valley Research Station	86.63	129.25	19.06	12.13	14.19	5.49

Table 4. Mean performance of cowpea M_6 experimental lines at three locations in Zimbabwe.

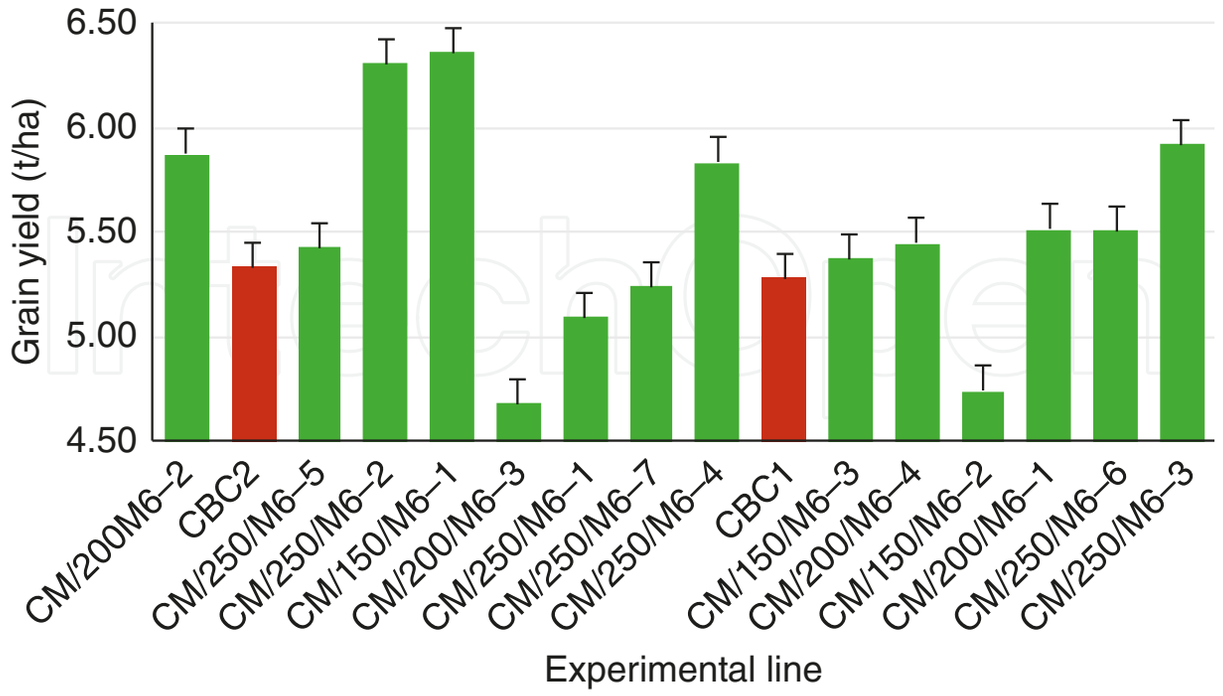


Figure 3. The grain yield of cowpea M_6 experimental lines at three locations in Zimbabwe.

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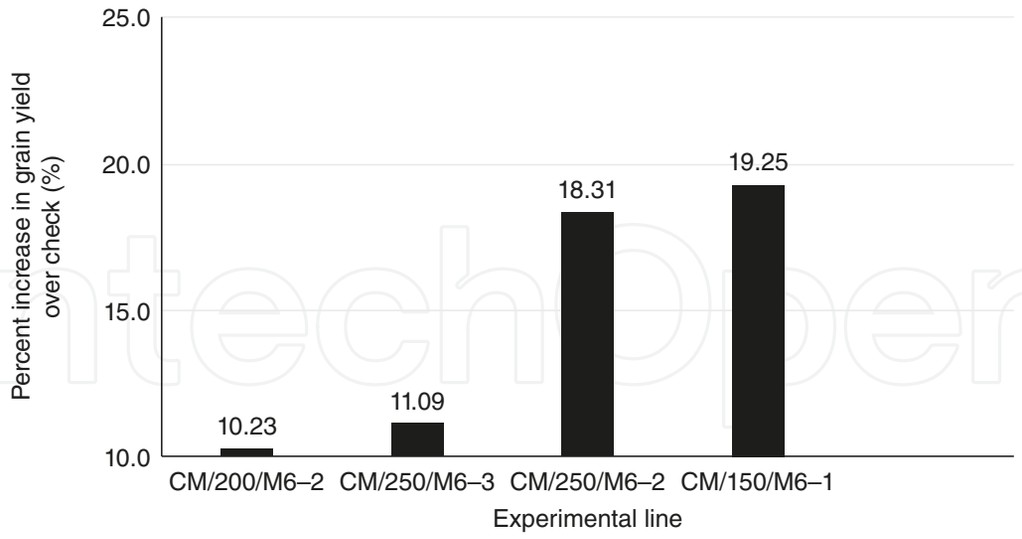


Figure 4. The yield advantage of the best four cowpea M_6 experimental lines over the check cultivar (CBC-2) that were evaluated at the Save Valley Research Station (Zimbabwe) during winter under irrigation.

3.3. Seedling performance of tepary bean mutants

In the study that evaluated the traits that are associated with seedling vigour in tepary bean, the %G declined with increased EMS dose in all the three genotypes, indicating that the chemical mutagen depressed seed germination in tepary bean; hence, the mutagenesis of tepary bean with EMS is unlikely to improve seed germination in the species. 'GEN-6' attained the highest (84.4%) seed germination at 0.5% EMS (v/v) while 'GEN-4' achieved the lowest (48.9%) seed germination at 2.0% EMS (v/v). The highest LD_{50} was estimated to be 3.37% EMS dose (v/v) for 'GEN-3' while 'GEN-6' achieved the lowest (2.26 % EMS dose v/v) (**Table 5**). This suggested that each genotype has a specific optimum dose that induces useful mutations that can be exploited in tepary bean breeding. In addition, the coefficients of determination for each of the linier functions were high (>75%), indicating that there was a notable association between the reduction in seed germination and the concentration of EMS.

Tepary bean genotype	Linear equation	LD_{50} (%EMS)
GEN-3	$y = -13.56x + 95.76$	3.37
GEN-4	$y = -15.98x + 92.88$	2.68
GEN-6	$y = -19.98x + 95.08$	2.26

Table 5. The LD_{50} of three tepary bean genotypes that were treated with ethyl methanesulphonate to induce mutation.

The shoot height (SHT) varied with EMS dose within the genotypes (**Figure 5**). In comparison with the seedlings of the control (0.0% EMS), the SHT in both 'GEN-3' and 'GEN-4' increased by more than 30.0% at the 0.5% EMS but decreased steadily thereafter. In contrast, the SHT in

'GEN-6' was reduced consistently between 0.5 and 1.5% EMS (v/v). Similar studies in other crops also revealed that seedling height decreased with increase in EMS dose [24,25]. With the exception of the PRL, there were significant ($P < 0.05$) differences due to dose effects among the seedlings in all the attributes that were evaluated (**Table 6**). The mean length of the primary roots among the seedlings was >40.0% of the SRL, but on average the SRL was markedly stimulated at 0.5% EMS (v/v).



Figure 5. Variation in the shoot height and the number of secondary roots of the tepary bean seedlings within genotypes. (The numbers in bold below each seedling represent the corresponding EMS dose treatment.)

%EMS dose (v/v)	%G	NSR	PRL	SRL	SHT
0.0	100.0 a	20.56 ab	60.22 a	23.22 bc	67.11 a
0.5	79.99 b	30.10 a	53.15 a	68.92 a	83.16 a
1.0	73.32 bc	29.97 a	77.30 a	26.11 b	58.00 ab
1.5	74.16 b	15.92 bc	52.74 a	19.18 bc	34.83 bc
2.0	60.00 c	8.34 c	20.37 b	6.89 c	26.08 c

Means followed by the same letter in each column are not significantly different ($P < 0.05$). [%G = percent seed germination; NSR = number of secondary roots; PRL = primary root length; SRL = secondary root length; SHT = shoot height].

Table 6. The main effects of ethylmethane sulphonate dose on five attributes of seedling vigour in tepary bean.

The number of secondary roots (NSR) generally increased by >45.0% at 0.5 and 1.0% EMS (v/v) dose. Both the maximum (39.0) and lowest (4.0) number of lateral roots were observed for

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'GEN-4' at 0.5 and 2.0% EMS (v/v), respectively. In addition, the untreated seedlings of 'GEN-4' developed 50.0% fewer secondary roots than the untreated seedlings of the other two genotypes but trebled the NSR on treatment with 0.5% EMS (v/v) (**Figure 6**). A highly significant ($P < 0.01$) positive linear relationship was observed between the PRL and the NSR. The NSR also showed a positive significant linear relationship with the SHT, indicating that the seedlings which produced many lateral roots also developed relatively tall shoots.

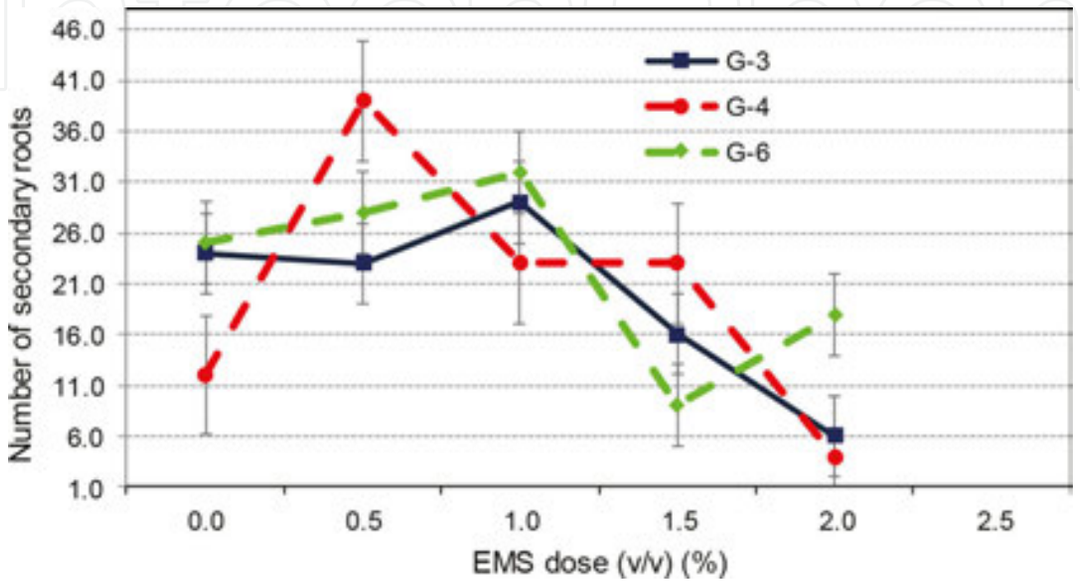


Figure 6. The effect of varying doses of ethyl methanesulphonate on the number of secondary roots in the seedlings of three tepary bean genotypes (G-3 = GEN-3; G-4 = GEN-4; G-6 = GEN-6).

3.4. Field performance of tepary bean mutants

At the adult plant stage, there was a significant ($P < 0.05$) influence of the mutant generation on the NPP, SDW as well as 100-SW. A significant effect of the mutant generation on the NPP was also reported previously in EMS derived horsegram genotypes [28]. The genotype significantly ($P < 0.05$) influenced the PL but not the seed size. Similarly, the dose of EMS showed significant ($P < 0.05$) on both the PL and the seed size. However, the seed size was relatively small (<10.0 g per 100 seeds) compared to the size reported for tepary bean in other studies [29].

Because of the limited number of tepary bean genotypes used in the study, it was difficult to make firm conclusions about its response to EMS, but the results suggested that the mutagen suppresses seed germination and can increase the number of secondary roots in this legume. Therefore, from a plant breeding point of view, the treatment of tepary bean seed with EMS is unlikely to improve seed germination but could increase the number of lateral roots. In previous studies, the number of lateral roots (or profuse branching) and the root length (or capacity for deep rooting) were associated with tolerance to drought in tepary bean or common bean [30–32].

4. Conclusions

These findings provide reference doses for large-scale gamma irradiation of cowpea as well as chemical mutagenesis for tepary bean. In addition, the germplasm produced from these approaches has the potential for selection in a range of agro-ecological conditions across the region, thus creating alternative cropping systems for the smallholder growers. The field trials under irrigation indicated that cowpea can be produced during the off-season, thus providing more options for legume farmers and enhancing food security in the region. In future, it will be interesting to investigate the impact of radiation on the nutritional attributes of these two legumes. In addition, a study of the genetic control of the sensitivity of cowpea to gamma radiation could provide valuable information about its genetic manipulation. The contrast in radio-sensitivity between the cowpea genotypes observed in this study could provide ideal parental combinations for generating segregating progenies for the genetic study of the trait. The effects of mutagenesis using either of the techniques on pest resistance (for instance, to weevils) in diverse germplasm of the two legumes could also be interesting.

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