



Research article

Impact of chemical mutagenesis using ethyl methane sulphonate on tepary bean seedling vigour and adult plant performance

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ABSTRACT

Tepary bean is an important food legume. The genetic improvement of the crop is limited by a narrow genetic base but this genetic base can be broadened through induced mutagenesis. This study was designed to determine the (i) effects of chemical mutagenesis using ethyl methane sulfonate (EMS) on M₁ seedlings (ii) the adult plant performance of M₂ - M₄ generations and (iii) the relationships between growth attributes at the seedling and adult plant stage. Mutagenized seed with varying doses of EMS was germinated at room temperature in order to raise M₁ seedlings. There were highly significant ($P < 0.01$) differences due to dose effects among the seedlings. The highest LD₅₀ (3.37 % EMS v/v) was observed for 'Genotype 3'. Under field conditions, all the three factors influenced the plant performance. The results demonstrated the potential of EMS to induce genotypic variation and desirable agronomic traits in tepary bean.

1. Introduction

Tepary bean (*Phaseolus acutifolius*; $2n = 2x = 22$) is a self-pollinating food legume which originated from the arid region of Mexico and the south-western United States of America (Garvin and Weeden, 1994). It is tolerant to drought (Porch et al., 2013). It is also useful in the genetic improvement of common bean (Muñoz et al., 2004; Singh and Munoz, 1999; Mejía-Jiménez et al., 1994; Pratt et al., 1990).

In Africa, tepary bean is cultivated in many countries including Benin (Dinghani et al., 2018), Botswana (Molosiwa et al., 2014), Kenya (Shisanya, 2003) and South Africa (Gwata et al., 2016). The production is constrained by the lack of improved cultivars that produce good grain yield under the dryland conditions. The smallholder growers utilize traditional varieties that are often poor in seed quality particularly germination which results in poor crop stands and diminished yields. The ability of the crop to withstand moisture stress was attributed partly to, profuse branching in the root system (Butare et al., 2011). Therefore, an evaluation of the traits associated with seedling vigour is useful in the genetic improvement of tepary bean. However, the genetic improvement for grain yield in tepary bean is limited by the narrow genetic base but mutation breeding offers an opportunity to increase the genetic variability for crop improvement (Oladosu et al., 2016). In particular, the use of ethyl methane sulphonate (CH₃SO₂OC₂H₅) (EMS) was considered

useful in mutation breeding (Girija and Dhanavel, 2009). In terms of safety, more than 2250 crop varieties have been developed worldwide using mutation approaches including EMS mutagenesis (Maluszynski et al., 2000) with no harmful effects on human health. Some of the mutation derived varieties showed enhanced quality in traits such as seed oil profile and reduced toxins, among many others (Ahloowalia et al., 2004). However, to date there is a paucity of information regarding for instance, the LD₅₀ in tepary bean which is necessary for comparing the relative effectiveness of different mutagenic treatments as well as indicating the optimum dose of the mutagen that induces useful agronomic traits (Mba et al., 2010; Tshilenge-Lukanda et al., 2012).

Field evaluation of adult plants is important in a crop improvement program. Although there are no reports of similar field evaluation work utilizing mutation breeding in tepary bean *per se*, the approach was applied successfully in mung bean (Khan and Goyal, 2009), common bean (Maluszynski et al., 2000), and soybean (Carroll et al., 1986). However, one of the drawbacks in this breeding approach is that the traits of interest may still be segregating in the early generations but in the case of mutant populations, the stability of the mutations is expected to increase with the generation.

The field production of tepary bean is generally similar in many parts of the world including Botswana (Molosiwa et al., 2014), Colombia (Rao et al., 2013), Kenya (Shisanya, 2005) and the United States of America

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(Bhardwaj et al., 2002). However, there are no reports of the field performance of early generation tepary bean mutants. In addition, there is insufficient information regarding the combined effects of the chemical mutagen (EMS) and the mutant generation in tepary bean. Although the use of EMS during the pre-breeding phase of a genetic improvement program is useful, one of its drawbacks is that it can induce a broad spectrum of mutations in plants due to the random nature of genomic mutations in the target species. Consequently, it is necessary to evaluate the mutants in order to identify the useful mutations. Therefore, the specific objectives of this study were to determine the (i) effects of chemical mutagenesis using ethyl methane sulfonate (EMS) on early generation seedling vigour (ii) field performance of the early segregating generations ($M_2 - M_4$) adult plants and (iii) the relationships between the growth attributes at both the seedling and adult plant stage among three tepary bean genotypes. The mutant germplasm showing potential for improved seedling vigour and agronomic performance could be used as a complementary tool in the genetic enhancement of tepary bean.

2. Materials and methods

2.1. Experiment #1: evaluation of M_1 seedling vigour

2.1.1. Genetic material and seed mutagenesis

The seed of each of three genotypes of tepary bean that were obtained originally from growers in Sekhukhune District (Limpopo Province, South Africa) was used in the study. These genotypes were self-fertilized over four cycles in order to optimize homozygosity prior to chemical mutagenesis. The selfing was conducted in the greenhouse at the study location at Thohoyandou (22° 58' S, 30° 26' E; 596 m a.s.l.) in Limpopo Province (South Africa). The seed coat in all the genotypes was white.

A sample of healthy clean seed (approximately 250) of each of three randomly selected tepary bean genotypes (M_0) was surface sterilized by soaking in 70.0% ethanol for 1 min and rinsing three times followed by soaking in 30.0% sodium hypochlorite bleach solution (2.0% 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 a specially designed sachet made of nylon mesh (measuring about 7.0 cm in width x 11.0 cm in length). The seed was transferred to aqueous solutions of varying doses of EMS (0.0, 0.5, 1.0, 1.5, 2.0 %; v/v) 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.

2.1.2. Seed germination and measurements

The mutagenized seed samples (M_1) from each of the three genotypes of tepary bean were germinated under laboratory conditions at room temperature in plastic jars (Figure 1) measuring 7.5 cm × 8.0 cm (diameter x height) in order to raise M_1 seedlings. Prior to germination, the inside of the base of each jar was lined with moist filter paper before placing 10–12 seeds and ensuring that each individual seed was free from other seeds in the jar.

At the initiation of the first trifoliate leaf (8 d after germination), the following seedling traits were measured:

- (i) percent seed germination (%G)
- (ii) primary root length (PRL) (mm)
- (iii) secondary root length (SRL) (mm)
- (iv) shoot height (mm) (SHT)
- (v) root dry weight (g) (RDW) and
- (vi) shoot dry weight (SDW) (g).

The root and shoot dry weights were obtained after drying (to a constant weight) separately the individual roots and shoots at 70.0 °C for two days.

2.1.3. Experimental design and data analysis

The experiment was laid out as a split plot design (SPD) arranged in a randomized complete block and replicated three times. Each genotype constituted the whole plot (factor A = 3) while the doses formed the sub-plot (factor B = 5). Each replication consisted of 15 jars (treatment combinations) to make a total of 45 jars. The data sets for each variable were analysed in SAS (SAS Institute, 2009) using the following statistical linear mixed model:

$$Y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \gamma_k + (\beta\gamma)_{jk} + \varepsilon_{ijk} \quad (1)$$

where:

Y_{ijk} = observation corresponding to k^{th} level of sub-plot factor (C), j^{th} level of main plot factor (B) and the i^{th} replication

μ = denotes general mean

α_i = denotes i^{th} block effect

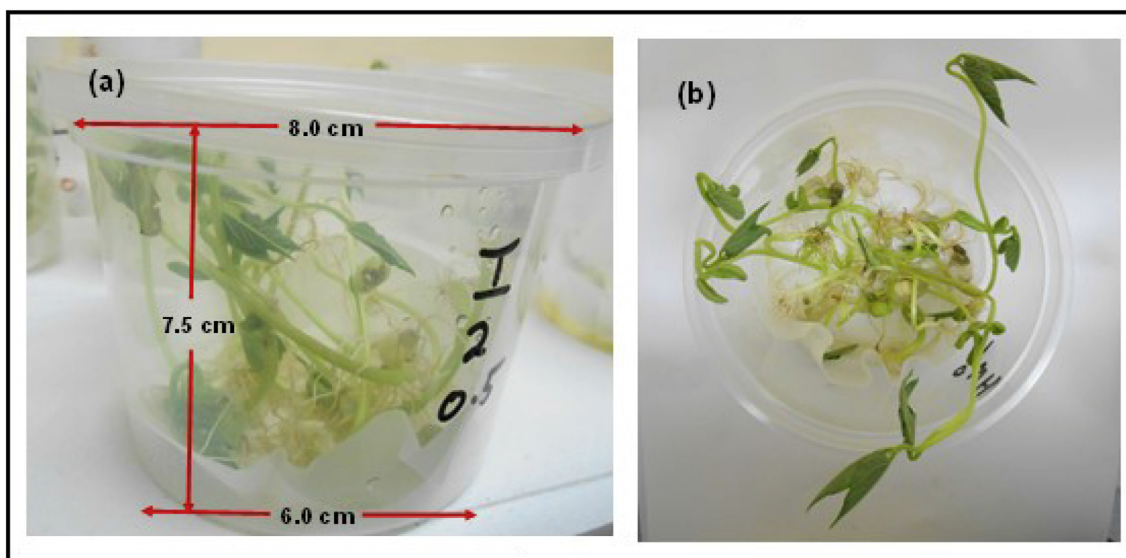


Figure 1. M_1 seedlings of tepary bean were raised in plastic jars (a) side view and (b) aerial view.

β_j = denotes j^{th} main plot treatment effect
 $(\alpha\beta)_{ij}$ = denotes interaction between j^{th} level of main-plot treatment and the k^{th} level of sub-plot treatment
 γ_k = denotes subplot treatment effect
 $(\beta\gamma)_{jk}$ = denotes k^{th} sub-plot treatment effect
 ε_{ijk} = denotes the error components.

$(\beta\psi)_{jk}$ = the fixed interaction effect between the j^{th} genotype and the k^{th} dose
 $(\alpha\beta\psi)_{ijk}$ = the random interaction effect between the i^{th} mutant generation, j^{th} genotype and k^{th} dose
 E_{ijkl} = the residual error

2.2. Experiment #2: evaluation of seedling vigour and agronomic performance of segregating early mutant generations ($M_2 - M_4$)

2.2.1. Genetic material and seed mutagenesis

The seed of three mutant populations (M_2 , M_3 and M_4) of tepary bean was derived from each of three different genotypes ('Genotype 3', 'Genotype 4' and 'Genotype 6') using varying doses of EMS as described above. The successive mutant generations (M_2 , M_3 and M_4) for each genotype (consisting of approximately 54 plants) were raised in the greenhouse at the study location by retaining a portion of the seed of each preceding generation in order to grow the next generation.

2.2.2. Seedling vigour and field measurements

In order to determine the seedling vigour, thirty randomly selected seeds of each mutagenized genotype per generation were germinated in the laboratory at room temperature in plastic jars as described above. The seedlings were allowed to grow for eight days after which five of the seedling vigour attributes (excluding percent germination) as described above (see experiment #1) were measured. For field evaluation, the mutagenized seed of each treatment combination (generation x genotype x dose) was planted separately in a single row field plot (Bhardwaj et al., 2002) measuring 3.0 m long and spaced at 0.6 m between adjacent rows. The seed was planted at 0.1 m within the row. The crop was raised under rain-fed conditions during the normal cropping season following standard tepary bean management practices throughout the season. During the reproductive stage or at harvest, the following agronomic attributes were measured:

- (i) canopy width (CW) at flowering
- (ii) plant height at the podding stage (PHT)
- (iii) pod length (PL)
- (iv) number of pods per plant (NPP)
- (v) podding score (PS)
- (vi) shoot dry weight (SDW)
- (vii) the number of seed per pod (NSP)
- (viii) number of primary branches per plant (NPB) and
- (ix) 100 seed weight (100-SW).

2.2.3. Experimental design and data analysis

In both the laboratory experiment (for determining seedling vigour) and the field trial (for adult plant evaluation), a $3 \times 3 \times 5$ (genotype x generation x EMS dose) factorial experiment arranged in randomized complete block design and replicated three times was used. The data sets of each quantitative variable were analyzed in SAS (SAS Institute, 2009) using the following statistical linear mixed model:

$$Y_{ijkl} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \psi_k + (\alpha\psi)_{ik} + (\beta\psi)_{jk} + (\alpha\beta\psi)_{ijk} + E_{ijkl} \quad (2)$$

where:

Y_{ijkl} = the observation for generation i of genotype j in dose k
 μ = the overall mean
 α_i = the random effect of the i^{th} mutant generation
 β_j = the random effect of the j^{th} genotype
 $(\alpha\beta)_{ij}$ = the random interaction effect between the i^{th} mutant generation and the j^{th} genotype
 ψ_k = the fixed effect of the k^{th} dose
 $(\alpha\psi)_{ik}$ = the random interaction effect between the i^{th} genotype and the j^{th} dose

3. Results

3.1. Seedling vigour of M_1 generation

There were highly significant ($P < 0.01$) differences due to dose effects among the seedlings in the majority of the attributes that were evaluated (Table 1). The primary roots in some of the treatments remained stunted and hence failed to develop secondary roots (Figure 2). 'Genotype 6' attained the highest (84.4%) seed germination at 0.5% EMS (v/v) while 'Genotype 4' achieved the lowest (48.9%) seed germination at 2.0% EMS (v/v) (Figure 3). Among the three genotypes, 'Genotype 6' appeared to be the least sensitive to the range of EMS doses that were used in the study. Nonetheless, %G declined with increased EMS dose among all the genotypes suggesting that EMS depressed seed germination in tepary bean. In comparison with the seedlings of the control (0.0 % EMS), the shoot height (SHT) in both 'Genotype 3' and 'Genotype 4' increased by more than 30% at the 0.5% EMS but decreased steadily thereafter (Figure 3).

The SDW showed an upward trend for all the genotypes over the whole range of EMS dose (Figure 4). However, at 2.0% EMS (v/v) the SDW had increased by at least two-fold compared to the SDW observed at 0.0% EMS dose (v/v) suggesting that ≤ 2.0 % EMS (v/v) doses of the chemo-mutagen improved the SDW. In all the three genotypes, the RDW showed an initial increment (at 0.5 % EMS v/v) but diminished steadily thereafter (Figure 4). On average, the roots of 'Genotype 6' were approximately 70.0% heavier than those for 'Genotype 4' (0.023g) at 1.0% EMS (v/v).

The response of the percent seed germination (%G) of 'Genotype 3' to EMS treatment was estimated by the linear function $y = -13.56x + 95.76$ (Figure 5). By using this equation, (at $y = 50.0\%$ germination), the LD_{50} for 'Genotype 3' was estimated to be 3.37% EMS dose (v/v). Similarly, the LD_{50} for 'Genotype 4' and 'Genotype 6' were estimated to be 2.68% and 2.26% EMS dose (v/v) respectively. In addition, there were high coefficients of determination for each of the linear functions ($>75\%$) suggesting that there was a notable association between the reduction in seed germination and the concentration of the mutagen.

There was a highly significant ($P < 0.01$) but negative linear relationship between the SDW and %G. In contrast, the NSR showed a positive significant linear relationship with the SHT indicating that the seedlings that produced many lateral roots also developed relatively tall shoots.

3.2. Seedling vigour of $M_2 - M_4$ generations

There were significant ($P < 0.05$) effects of the mutant generation on both the SHT and NSR. Highly significant ($P < 0.01$) interaction effects between generation x genotype, generation x dose, genotype x dose, and generation x genotype x dose were observed for SDW. Similarly, there were highly significant ($P < 0.01$) effects in the generation x dose and genotype x dose interactions for RDW and PRL respectively. The mean NSR was 19.56. On average, the SHT was double the PRL while the SDW was ten-fold heavier than the RDW. Significant differences ($P < 0.05$) among the mutant generations were observed for SHT, NSR and SDW. In comparison with the M_2 generation, the M_4 generation showed 17.32% reduction in SHT. In contrast, the mean NSR in M_3 increased by 33.20% over the M_2 generation. The RDW in 'Genotype 3' was 30.0% heavier than that of each of the other two genotypes. The heaviest shoots were induced at 0.5% EMS (v/v) while the lightest shoots were observed for the 0.0% EMS (v/v) (or control) suggesting that chemical mutagenesis

Table 1. Analysis of variance for seven attributes of seedling vigour in M_1 seedlings among three tepary bean genotypes. (%G = percent seed germination; NSR = number of secondary roots; PRL = primary root length; SRL = secondary root length; SHT = shoot height; RDW = root dry weight; SDW = shoot dry weight).

| Source | df | Mean Square | | | | | | |
|--------------------|----|--------------|-------------|--------------|--------------|--------------|--------------------------|--------------------------|
| | | %G | NSR | PRL | SRL | SHT | SDW ($\times 10^{-4}$) | RDW ($\times 10^{-4}$) |
| Replication (R) | 2 | 987.7622 * | 15.8735 | 91.9677 | 16.8075 | 49.7076 | 1.4 | 1.3 |
| Genotype (G) | 2 | 274.5298 | 44.2002 | 199.9726 | 85.4167 | 693.2509 | 0.6 | 1.9 |
| R x G | 4 | 91.5242 | 52.3545 | 470.7139 | 369.5702 | 184.8555 | 2.2 | 0.2 |
| Dose (D) | 4 | 1679.5891 ** | 712.3444 ** | 3840.6172 ** | 4997.0008 ** | 4925.6741 ** | 9.2 | 7.3 |
| G x D | 8 | 92.0413 | 186.0933 * | 1600.7159 ** | 144.1632 | 517.9523 | 2.1 | 1.0 |
| Mean | | 77.56 | 20.58 | 52.75 | 28.87 | 53.93 | 0.0739 | 0.0068 |
| R ² (%) | | 74.74 | 74.66 | 72.94 | 86.58 | 74.48 | 48.92 | 86.78 |
| C.V. (%) | | 17.05 | 41.56 | 41.24 | 42.05 | 35.76 | 23.02 | 25.27 |

**Significant at the 1.0% probability level; * Significant at the 5.0% probability level.



Figure 2. Some seedlings [(a), (c) and (d)] failed to develop roots and remained stunted while others (b) were normal.

(up to 2.0% EMS (v/v)) stimulated dry matter accumulation in the shoots of the mutant plants.

3.3. Adult plant performance of M_2 – M_4 generations

During the generation advancement from M_1 to M_2 , some individual genotypes segregated for the stay-green trait and early maturity (Figure 6). In addition, some genotypes developed a relatively high number of pods (Figure 7). Plant height also varied markedly.

The SDW decreased markedly (by >25.0%) as the mutant generations advanced from M_2 to M_4 but five of the attributes (PHT, CW, PL, NPP and NSP) remained unchanged over the three generations for some of the traits such as PHT and NSP (Table 2). Interestingly, the seed size as measured by 100-SW was similar among the three genotypes. There were

highly significant ($P < 0.01$) positive correlations between CW and each of the attributes that was measured (Table 3). Similarly, there was a highly significant ($P < 0.01$) positive correlation between the NPP and 100-SW.

4. Discussion

The results of this study were largely in agreement with the findings that were reported for other field crops that were treated with EMS (Bolbhat and Dhumal, 2012; Khan and Goyal, 2009). The response of the three genotypes to EMS in terms of %G was similar but the dose effects varied significantly. Because of the limited number of genotypes used in the study, it is difficult to make unequivocal conclusions about the response of tepary bean to EMS but the results suggested that the mutagen suppresses seed germination 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. The evaluation of %G provided an insight into the variation in genotypic sensitivity to the EMS concentration as demonstrated by the differences in the LD_{50} among the genotypes. ‘Genotype 3’ appeared to tolerate higher doses of EMS than the other two indicating differential response to EMS among the genotypes. The observed variation in seed germination among the genotypes could be attributed probably to differential sensitivity to the mutagen with the least sensitive genotype exhibiting a relatively higher percent germination. The absorption of mutagens can be influenced by various factors such as the water content in the tissues of the seed (Boros and Wawer, 2018; Ke et al., 2019). The seeds of the different tepary bean genotypes probably varied in their morphological attributes such as size, seed coat thickness and hydration capacity which could have influenced the rate of absorption of the mutagen, hence its genetic effects. In general, the mutations tend to stabilize as the generations increase such that selection of potential crop cultivars starts at about the M_6 or higher (Kato et al., 2020) after allowing for sufficient segregation of homozygotes and the reduction of heterozygotes through successive cycles of self-pollination. On the genetic level, EMS induces point mutations resulting in new or modified gene products (that is, proteins) which may include the various hydrolytic enzymes that are involved in seed germination or plant growth (Lehmann and Ratajczak, 2008) as observed in the study. The LD_{50} values that were determined for the three genotypes also provided new information regarding the optimum EMS dose for tepary bean. By interpolation, it would be reasonable to assume that EMS doses >10.0% (v/v) could induce lethal mutations and thus stop seed germination completely. Nonetheless, a firm conclusion regarding this observation will require empirical evidence from large-scale seed mutagenesis of diverse tepary bean germplasm. In addition, these differences in the LD_{50} among the genotypes also suggested that it is more accurate to determine the range of optimum doses of the EMS instead of a single fixed value for all the genotypes within tepary bean.

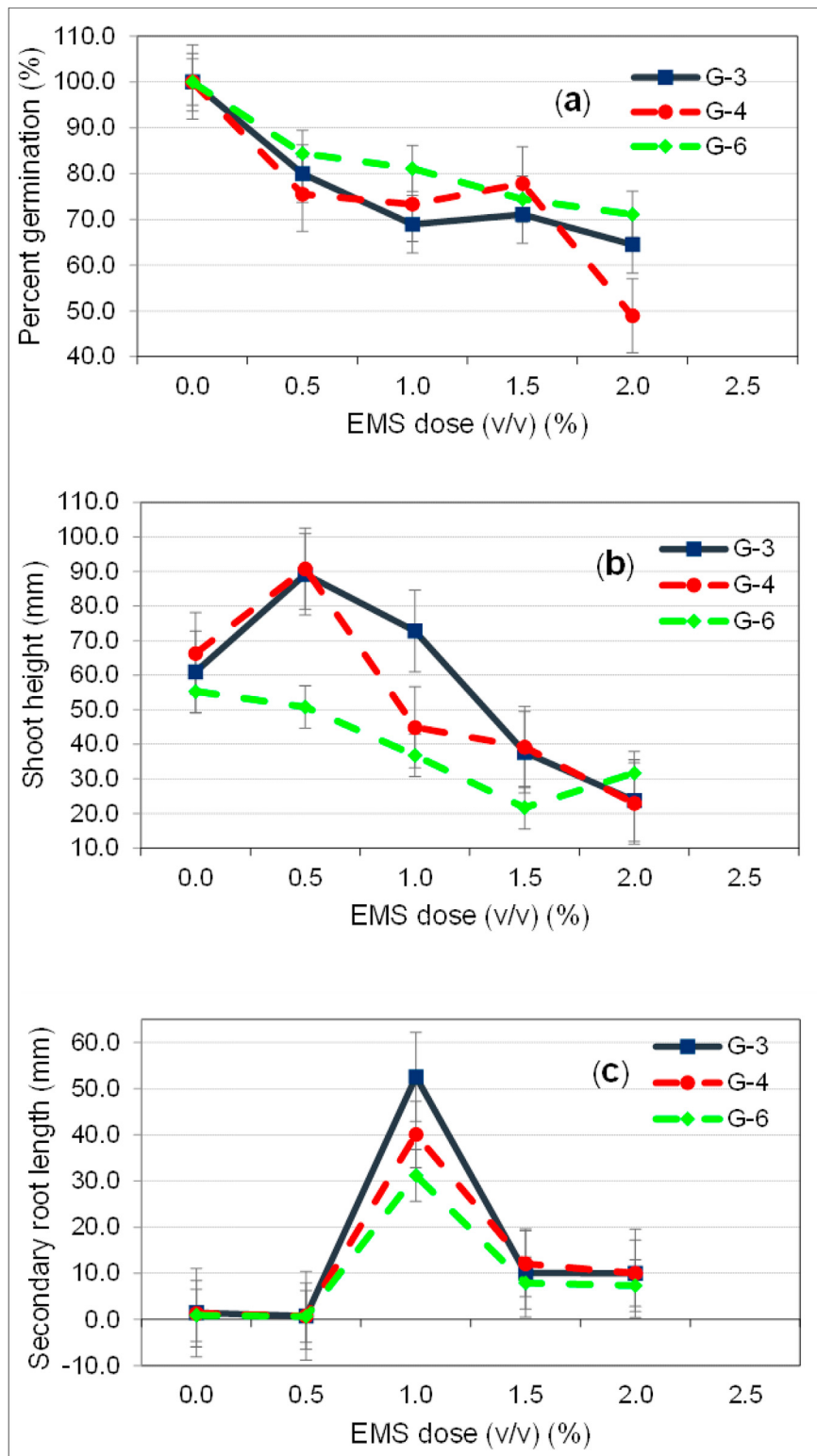


Figure 3. The effect of varying doses of ethyl methane sulphonate on (a) percent germination (b) shoot height and (c) secondary root length in three tepary bean genotypes. (G-3 = Genotype 3; G-4 = Genotype 4; G-6 = Genotype 6).

The significant positive relationship between NSR and PRL was potentially useful in the sense that at the field level, genotypes with long (deep) roots and profuse root branching can be expected to withstand lodging for instance (Butare et al., 2011). The stunted growth of some seedlings (particularly the primary roots) due to EMS indicated that percent seed germination *per se* is insufficient to evaluate the impact of the mutagen on the species. The evaluation of the seedling vigour traits

such as root length and root branching were useful in evaluating the impact of EMS mutagenesis on tepary bean. However, in comparison with the laboratory conditions, the expression of most of the phenotypic traits at the field level under rain-fed conditions, would be influenced relatively more by the natural variation and fluctuation of environmental factors such as soil moisture distribution, pH as well as temperature. In contrast, the variation in these factors was maintained at a

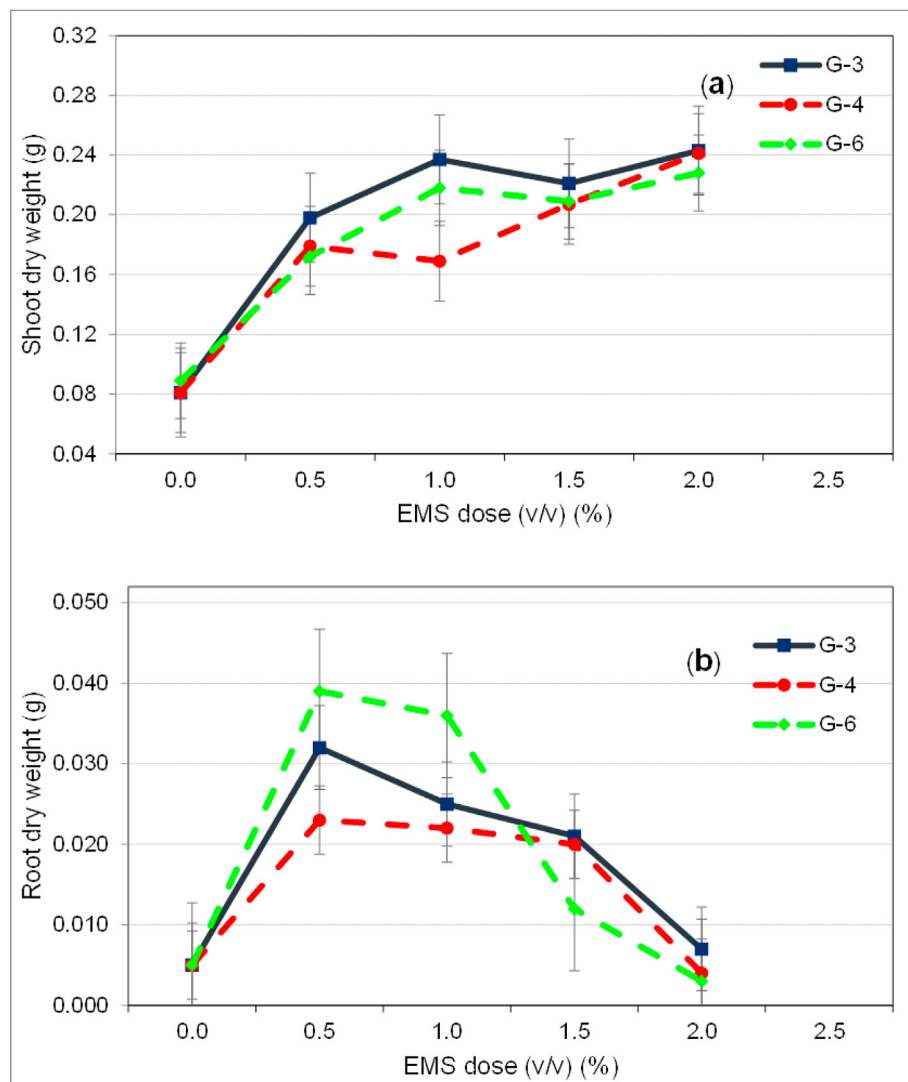


Figure 4. The effect of varying doses of ethyl methane sulphate on (a) shoot dry weight and (b) root dry weight in three tepary bean genotypes. (G-3 = Genotype 3; G-4 = Genotype 4; G-6 = Genotype 6).

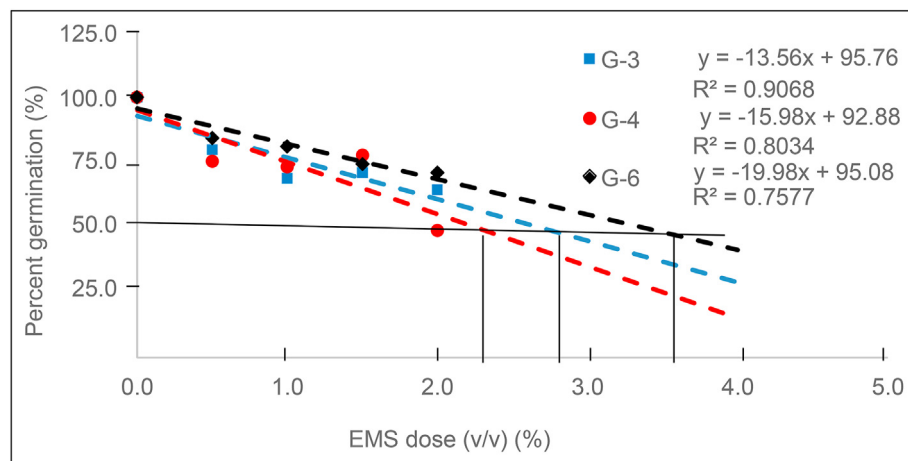


Figure 5. Percent germination and fitted straight lines to estimate the LD₅₀ in three tepary bean genotypes that were treated with varying doses of ethyl methane sulphate. (G-3 = Genotype 3; G-4 = Genotype 4; G-6 = Genotype 6).

minimum in the laboratory. Therefore, in this regard, the field results could be interpreted as more reliable. Nonetheless, despite a low correlation that may exist between the two sets of the results, both

laboratory and field approaches have been recommended widely in similar agricultural studies (Hohmann et al., 2016; Poorter et al., 2016; Stevens et al., 2008).



Figure 6. Individual mutant plants segregated for (a) the stay-green trait and earliness and (b) late flowering in the M₂ generation.



Figure 7. Individual mutant plants segregated for the number of pods in the M₃ generation.

Table 2. Mean performance of adult plants among three tepary bean genotypes over three mutant generations. (SHT = shoot height; PRL = primary root length; NSR = number of secondary roots; SDW = shoot dry weight; RDW = root dry weight).

| Factor | PHT(cm) | CW(cm) | PS | SDW(g) | PL (cm) | NPP | NSP | NPB | 100-SW(g) |
|-------------------|---------|---------|--------|---------|---------|---------|---------|--------|-----------|
| Generation | | | | | | | | | |
| 2 | 42.36 a | 27.00 a | 2.17 b | 10.67 a | 6.33 a | 15.93 a | 4.44 a | 5.14 a | 7.87 b |
| 3 | 45.78 a | 28.42 a | 3.21 a | 8.97 ab | 6.41 a | 17.66 a | 4.60 a | 5.15 a | 9.32 a |
| 4 | 43.23 a | 25.58 a | 2.36 b | 7.86 b | 6.31 a | 13.08 a | 4.60 a | 4.31 b | 8.55 ab |
| Genotype | | | | | | | | | |
| G-3 | 40.49 a | 26.27 a | 2.46 a | 8.28 a | 6.14 b | 13.78 a | 4.36 b | 5.00 a | 8.10 a |
| G-4 | 46.20 a | 27.36 a | 2.59 a | 10.19 a | 6.47 a | 16.43 a | 4.50 b | 4.94 a | 8.70 a |
| G-6 | 44.77 a | 27.38 a | 2.76 a | 9.0 a | 6.45 a | 16.44 a | 4.78 a | 4.68 a | 8.99 a |
| Dose | | | | | | | | | |
| 0.0 | 44.31 a | 28.26 a | 2.67 a | 7.77 a | 6.49 a | 15.89 a | 4.71 a | 4.72 a | 8.84 ab |
| 0.5 | 45.80 a | 28.67 a | 3.00 a | 10.34 a | 6.37 ab | 17.30 a | 4.50 ab | 4.89 a | 9.66 a |
| 1.0 | 42.17 a | 25.85 a | 2.50 a | 9.28 a | 6.40 ab | 13.18 a | 4.51 ab | 4.93 a | 7.41 b |
| 1.5 | 44.80 a | 28.15 a | 2.44 a | 9.86 a | 6.33 ab | 17.50 a | 4.70 a | 5.23 a | 9.51 a |
| 2.0 | 41.86 a | 23.96 a | 2.42 a | 8.50 a | 6.16 b | 13.82 a | 4.32 b | 4.58 a | 7.50 b |

Means followed by the same letter in a column are not significantly different ($P < 0.05$).

The study also showed that a simultaneous evaluation of the mutant generations was useful in order to determine the stability of the agronomic traits over successive generations. Previous studies in other legumes including horsegram (Bolbhat and Dhupal, 2012) and adzuki

bean (Wu et al., 2010) also evaluated more than one mutagenized generation simultaneously. The results also showed that the change in the mutant generation influenced only some of the traits measured among the seedlings. For instance both the PRL and RDW did not change

Table 3. Coefficients of correlation (r) among nine attributes of mutant tepary bean seedlings that were evaluated under laboratory conditions at Thohoyandou (Limpopo Province, South Africa). (PHT = plant height; CW = canopy width; PS = pod score; SDW = shoot dry weight; PL = pod length; NPP = number of pods per plant; NSP = number of seeds per pod; NPB = number of pods per branch; 100-SW = weight of 100 seeds).

| | PHT | CW | PS | SDW | PL | NPP | NSP | NPB | 100-SW |
|--------|-----------|----------|----------|----------|----------|----------|----------|--------|--------|
| PHT | 1.0000 | | | | | | | | |
| CW | 0.7325** | 1.0000 | | | | | | | |
| PS | 0.5221** | 0.6308** | 1.0000 | | | | | | |
| SDW | 0.7383** | 0.6965** | -0.2366* | 1.0000 | | | | | |
| PL | 0.5217** | 0.4692** | 0.0000 | 0.4651** | 1.0000 | | | | |
| NPP | 0.6791** | 0.6356** | -0.1140 | 0.6800** | 0.6151** | 1.0000 | | | |
| NSP | 0.2468* a | 0.2759** | 0.1552 | 0.1407 | 0.6412** | -0.4586* | 1.0000 | | |
| NPB | 0.8341** | 0.7635** | 0.4875** | 0.7853** | 0.397** | 0.6454** | -0.0100 | 1.0000 | |
| 100-SW | 0.4452** | 0.4754** | 0.0980 | 0.2678** | 0.3650** | 0.5740** | 0.3186** | 0.0980 | 1.0000 |

** , * = highly significant and significant at the 1.0% and 5.0% probability levels respectively.

significantly with the generation suggesting that both of these parameters were stable over the three mutant generations (M_2 to M_4). In contrast, the NSR was significantly influenced by the mutant generation yet this trait was similar among the three genotypes. The significant generation \times genotype \times dose interaction for SDW suggested that the selection for this attribute should take into account all the three factors. In other words, the SDW of the best genotype in a given mutant generation should be determined on the basis of the combined effects of the EMS dose as well as the genotype. The similarity in the NSR that was observed among the genotypes and over the EMS dose range strongly suggested that the trait is highly conserved in tepary bean. Profuse branching of the roots was previously associated with drought tolerance in tepary bean (Butare et al., 2011). In this study, the consistency of the PRL and NSR among the genotypes was in agreement with the observations that profuse branching of roots (Butare et al., 2011) and deep rooting (Beebe et al., 2013) contribute to drought tolerance in tepary bean. If this is true, it suggests that the mechanisms of drought tolerance stabilize in the early generations of tepary bean.

The deferential development of the NPB between the fourth generation and the other two suggested that probably this trait cannot be selected for in the early generations since it showed instability over the three mutant generations in the study. This instability could be attributed to genetic rearrangements that are associated with meiotic events in the nuclear DNA of the mutant germplasm (Haughn and Somerville, 1987). In other legume species such as mungbean (Khan and Goyal, 2009) that were treated with EMS, similar instability in the number of fertile branches and pod load per plant of the early mutant generations were reported.

5. Conclusions

The LD₅₀ differed among the tepary bean genotypes indicating that a wide range of EMS dose can be applied in situations where diverse germplasm of the species is intended for chemo-mutagenesis. In addition, all the three factors namely the mutant generation, genotype and dose of the mutagen influenced the field performance of tepary bean mutants. Nonetheless, evaluation of mutant genotypes using both the laboratory and field conditions was useful since it allowed for testing a broad spectrum of traits that are commonly used in plant breeding programs. Future research work could utilize more test locations for the field evaluation of the agronomic attributes especially after advancing the germplasm further by two or more generations. Individual genotypes that showed improvements in yield components such as early maturity of pod yield could be isolated for further evaluation. The productivity of the selected germplasm after seed inoculation with rhizobia at planting could also be interesting.

Declarations

Author contribution statement

Andries Thangwana: Performed the experiments Eastonce T. Gwata: Conceived and designed the experiments; Wrote the paper.

Marvelous M. Zhou: Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

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Data availability statement

Data associated with this study has been deposited at The University of Venda Library under the accession number Thangwana A.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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