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Multivariate Characterization of Gamma-induced Phenotypic Variation in Sweetpotato (*Ipomoea batatas* L. Lam) Supports Putative Mutant Selection across Three Vegetative Generations

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Abstract

Sweetpotato (*Ipomoea batatas* L.) is a multi-purpose, high yielding major crop with wide agro-ecological adaptability. However, its improvement under climate change is limited by a narrow genetic base and breeding limitations that affect the development of resilient high yielding cultivars. Gamma-induced mutagenesis provides an alternative approach to generating phenotypic variation and the expanding pool of materials available for selection. This study aims to use gamma irradiation in three sweetpotato varieties to investigate phenotypic variation among putative mutant lines. A pot experiment was conducted in shade-house across three successive vegetative generations in 2023 and 2024 at Hawassa University, Ethiopia. Vine cuttings were subjected to three gamma-radiation doses (15, 25, and 35 Gy) from ^{60}CO source and 0 Gy doses as the control. Radio-sensitivity test showed varietal differences, with LD_{50} values estimated at 30.43 Gy for Alamura, 31.74 Gy for Kabode, and 33.58 Gy for Awassa-83. Putative mutant lines showed considerable phenotypic variability for qualitative and quantitative traits including plant type, leaf shape, lobe type, vine length, chlorophyll content, chlorophyll fluorescence, and tuber number. The first four principal components together explained 82.26%, 82.19%, and 80.18% in M1V1, M1V2, and M1V3, respectively, with morphological and physiological traits contributing to the observed multivariate differentiation. Cluster analysis grouped the putative mutant lines into five, four, and four clusters in M1V1, M1V2, and M1V3, respectively. Broad-sense heritability estimates were generally high for the quantitative traits evaluated, particularly in the later generations. Overall, substantial phenotypic variation was observed among plants derived from gamma-irradiated vine cuttings, while multivariate analysis provided an effective framework for characterizing the variation and supporting selection of putative mutant lines for subsequent evaluation in sweetpotato breeding.

Keywords: Sweetpotato, gamma irradiation, phenotypic variation, putative mutant lines, Multivariate analysis

1. INTRODUCTION

Sweetpotato is a starchy food crop of the Morning Glory family with chromosomal number 2n = 6x = 90 [1]. It is the important source of protein, vitamins, and carbohydrates used in raw, food, and feed products worldwide. It is a food and nutrition security crop feeding more than 100 million people in sub-Saharan Africa and Asia with global production exceeding 90 million tons annually [2, 3]. The national production area and quantity of sweetpotato is estimated over 62,000 ha [4] with production of 1,598,838 tons which is well below the potential yield under improved management [5].

It can grow in a variety of conditions and has a high potential yield [6]. The sweetpotato productivity is highly impacted by several abiotic factors such as drought, salinity, and extreme temperature and biotic factors including pathogens and pests. Moreover, the lack of improved varieties negatively affects productivity of the crop. However, conventional sweetpotato breeding is challenging because of its high heterozygosity, polyploid genome complexity, and self- and cross-incompatibility, and predominant vegetative propagation [7, 8]. Additionally, the absence of wild relatives and a narrow genetic base in Ethiopia further limited the progress in conventional crop improvement. The traditional crop improvement program of sweetpotato in Ethiopia contributed significantly to the present improved varieties, resulting in more than 30 varieties, including six orange-fleshed sweetpotato [9, 10].

However, the low root dry matter content of the old OFSP varieties and decline in the yield and resilience of the old varieties had significantly impacted the distribution and acceptance by end users, especially the farmers [10]. Hence, there is a need to facilitate the acceleration of sweetpotato improvement in Ethiopia, which is established in the recent national potato and sweetpotato development strategy [10]. Despite the importance of sweetpotato in Ethiopia, limited studies have evaluated the gamma-induced mutant population using a multivariate approach across successive generations. So the application of induced mutation is an alternative and effective tool to create diversity and accelerate sweetpotato improvement by generating heritable variation to develop new traits of interest in crops such as sweetpotato. Studies showed that mutation breeding is an effective tool for clonally developed plants [11], which is responsible for increasing genetic variation for different morpho-physiological characters in horticultural plants [12].

Mutation induction can be done through several techniques including chemicals (ethyl methanesulfonate (EMS)) and physical mutagenic agents such as non-ionizing radiation (UV), ionizing radiation (X-rays and gamma rays), and corpuscular radiation (protons, neutrons, alpha, and beta particles) [13, 14]. The ionizing irradiation such as gamma irradiation is the most widely used mutagen that can cause various genetic variations through direct and indirect DNA damage [15, 16, 17].

Several studies have shown that gamma irradiation causes larger number of small mutations and single-base substitutions are the predominant types of mutations as compared to EMS and carbon ion irradiation [16, 18, 1]. This leads to changes in phenotypic traits [19] that can be agronomically important such as regeneration percentage, days to flowering, number of leaf lobes, branch number, vine length, and chlorophyll content, days to sprouting, flowering time, plant height, and leaf color as well as yield [17, 20]. The report from the FAO/IAEA joint division, the Mutant Variety Database (MVD) shows more than 60% of the mutant varieties registered in MVD are the result of gamma irradiation [3].

The magnitude of the response depends on factors such as radiation dose, genotype, plant material, and experimental conditions. Therefore, determining an appropriate radiation dose and subsequently characterizing the resulting population are important steps in mutation-breeding programs.

Phenotypic characterization of induced populations across successive generations is particularly important because mutation breeding requires the identification and advancement of individuals with desirable characteristics. Descriptive statistics can quantify the magnitude of variation, while estimates of genotypic and phenotypic variability, heritability, and genetic advance can provide information relevant to selection. In addition, multivariate techniques such as principal component analysis (PCA) and hierarchical cluster analysis

(HCA) can summarize relationships among multiple traits and group entries according to their overall phenotypic profiles [21].

Despite the importance of sweetpotato improvement in Ethiopia, relatively limited information is available on the phenotypic characterization and multivariate-based selection of gamma-irradiated sweetpotato populations across successive vegetative generations. A systematic evaluation of variation generated by gamma irradiation can therefore provide useful information for identifying promising putative mutant lines and improving the efficiency of subsequent selection.

Therefore, the present study aimed to: (a) determine the radio sensitivity and LD_{50} of the three sweetpotato varieties following gamma-irradiation; (b) characterize the qualitative and quantitative phenotypic variability among putative mutant lines across three successive vegetative generations; (c) estimate components of genotypic and phenotypic variation, broad-sense heritability, and genetic advance for quantitative traits; and (d) use principal components and hierarchical cluster analyses to characterize multivariate phenotypic relationships and support the selection and advancement of putative mutant lines.

2. Material and Methods

2.1. Study area and experimental materials

Pot experiment was carried out under shade-house conditions at College of Agriculture, Hawassa University, Ethiopia. The study was conducted from 2023 to 2024 in three successive vegetative generations. Three released sweetpotato varieties Alamura, Kabode, and Awassa-83 were used in this study. The planting materials were obtained from the South Agricultural Research Institute (SARI), Ethiopia with permissions from the SARI administration. Information on varietal identity and background was cross-checked against available published literature and the International Potato Center (IPC) Sweetpotato catalogue [22], where applicable. However, herbarium voucher could not be obtained.

Vine cuttings obtained directly from the SARI, Ethiopia, were used as planting materials for the gamma irradiation treatments. The varieties were selected based on their nutritional value, yield potential and farmer preference. Varieties used in this experiment are described in Table 1.

Table 1: Description of sweetpotato varieties mutated

Variety	Maturity (days)	Flesh color	Years of release	Origin	Link
Alamura	120	Orange fleshed	2007	Ethiopia	https://sweetpotato-catalogue.cipotato.org/sweetpotato_variety/alamura/?utm_source=chatgpt.com
Kabode	120	Cream to Yellow	2007	Uganda	https://sweetpotato-catalogue.cipotato.org/sweetpotato_variety/kabode/?utm_source=chatgpt.com
Awassa-83	150	White fleshed	1983	Ethiopia	https://sweetpotato-catalogue.cipotato.org/global-search/?utm_source=chatgpt.com

Source: MACVRB (1983-2017)

2.2. Gamma irradiation treatment and LD_{50} experiment

Three varieties (Alamura, Kabode, and Awassa-83) were selected for the irradiation treatment having 30-40 cm length with four node healthy cuttings. For each variety 50 cuttings were prepared four irradiation treatments consisting 0, 15, 25, and 35 Gy giving 200 cuttings for each variety and total of 600 cuttings for all varieties. The cuttings were transported to Animal Health Institute Kality Tsetse Fly Research Center in Addis Ababa, Ethiopia for irradiation. Cuttings assigned to 15, 25, and 35 Gy were exposed to respective gamma irradiation dose from radioisotope ^{60}CO source. Exposure times were 45, 78, and 111 seconds, respectively. The 0 Gy treatments were maintained as non-irradiated control. Following the irradiation all 600 cuttings were planted under shade-house condition for subsequent evaluation. These doses were selected based on preliminary regeneration capacity tests, which identified 15 Gy as the ideal dose for maximum sprouting and 35 Gy as a high-inhibition threshold [6].

Following planting, emergence was monitored daily from 7 to 30 days after planting. Survival rate was defined as the proportion of cuttings that produced a visible shoot ≥ 1 cm within 30 days after planting [23]. Survival was recorded separately for each individual cutting and expressed as binary response, were 1 = survived, 0 = dead.

For each variety, a separate binomial logistic regression model was fitted, with gamma-radiation dose (Gy) as the explanatory variable and survival status as binary response. The logistic model was expressed as:

$$\text{logit}(p) = \beta_0 + \beta_1 (Dose)$$

.where *P* represents the probability of survival. The LD_{50} was estimated as the radiation dose corresponding to a predicted survival probability of 0.50:

$$LD_{50} = -\frac{\beta_0}{\beta_1}$$

The 95% confidence intervals (CI) of estimated LD_{50} values were calculated for each variety. The resulting dose response curves were used to visualize the relationship between gamma-radiation dose and survival probability.

Following the survival assessment, 293 surviving irradiated cuttings were retained as putative mutant lines. Among the surviving non-irradiated cuttings, the three original varieties were retained as control varieties for subsequent evaluation. Thus, the M1V1 population comprised 296 unique entries, consisting of 293 putative mutant lines and three control varieties. The M1V1 population was evaluated using an augmented randomized complete block design with 10 blocks. The 293 putative mutant entries were randomly assigned among the blocks as unreplicated test entries, while the three control varieties were included once in each block as replicated checks. Consequently, the M1V1 evaluation comprised 293 putative mutant plots and 30 control plots, giving a total of 323 plots. Phenotypic traits were evaluated in the M1V1 population and analyzed using principal component analysis (PCA) and hierarchical cluster analysis to characterize the overall phenotypic variation and group entries according to similarities in the trait performance. Clusters exhibiting relatively higher and desirable values for the evaluated phenotypic traits were identified, and the putative mutant entries belonging to the selected clusters were advanced to the next vegetative generation. Based on this selection procedure, 254 putative mutant entries were retained for M1V2, while all three control varieties were retained as checks.

In the second generation (M1V2), 254 putative mutant lines and 3 control varieties were evaluated using augmented RCBD with 9 blocks. The 254 putative mutant entries were randomly assigned among the blocks as unreplicated test entries, while each of the three control varieties was included once in every block as a replicated check. Thus, the M1V2 evaluation comprised 254 putative mutant plots and 27 control plots, giving a total of 281 plots. The phenotypic traits evaluated in M1V2 were subjected to PCA and HCA using the same procedure applied to M1V1. Based on multivariate characterization, 175 putative mutant entries were selected for advancement to M1V3, while the three control varieties were retained as checks.

The M1V3 population consisted of 175 selected putative mutant entries and the three control varieties. The population was evaluated using an augmented RCBD design with 6 blocks. The 175 putative mutant entries were randomly assigned among the 6 blocks as unreplicated test entries, while each of the three control varieties was included once in every block as a replicated check. Thus, the M1V3 evaluated comprised 175 putative mutant plots and 18 control plots, giving a total of 193 plots. Phenotypic traits were evaluated, and the resulting data were analyzed using PCA and hierarchical cluster analysis (HCA) to characterize the phenotypic variation and multivariate relationships among the putative mutant entries and control varieties (Refer Table S1 for experimental design summary per generations).

Putative mutant lines derived from the three sweetpotato varieties, together with their respective controls, were analyzed jointly to evaluate the overall spectrum of gamma irradiation associated phenotypic variability across different genetic backgrounds under a common experimental environment. Mutant-derived plants were advanced through successive vegetative generations, and clusters showing relatively desirable overall phenotypic performance were selected at each vegetative generation based on morpho-physiological traits. Selected plants were subsequently propagated and evaluated in the next generation.

2.3. Agronomic Procedures

The irradiated and control sweetpotato vine cuttings were planted at a depth of 10 cm in a 5 L volume pot filled with growth medium consisting of soil, sand and compost mixed at a ratio of 2:1:1 (v/v/v). The experiment was conducted under shade-house conditions. Before planting, the growth medium was irrigated with tap water to approximately 80% of field capacity and allowed to acclimate for 7 days. Morpho-physiological traits were recorded from 2 to 8 weeks after planting. All agronomic activities including weeding and pest management were applied based on the recommendation of Markos and Loha [24]. The experiment was conducted across three successive vegetative generations over a period of 12 months. The morpho-physiological data were collected from individual plants across all vegetative generations.

2.4. Data Collected

2.4.1. Dose response and lethal dose determination

Survival data were collected for all irradiated and non-irradiated cuttings following planting. Emergence was monitored daily from 7to 30 days after planting. A cutting was recorded as survival when it produced a visible

shoot ≥ 1 cm within 30 days after planting [23]. The survival status of each cutting was recorded as either survived or dead for each combination of sweetpotato variety and gamma-radiation dose. Survival counts and percentages were subsequently used to summarize the response of each treatment combination. The following formula was used to define the proportion of sprouting and survival percentage:

$$Sprouting\ \% = \frac{Number\ of\ sprouted\ plant}{Total\ number\ of\ vine\ planted} * 100$$

$$Survival\ \% = \frac{Number\ of\ survived\ plant}{Total\ number\ of\ vine\ planted} * 100$$

$$\%\ Reduction\ over\ control = (\%\ vine\ survival\ of\ the\ control) - (\%\ vine\ survival\ of\ the\ irradiated\ plant)$$

2.4.2. Morpho-physiological parameters

Morpho-physiological characterization of putative mutant lines and control plants were conducted across three successive vegetative generations to evaluate phenotypic variation induced by gamma irradiation. Qualitative traits were assessed following the standard sweetpotato descriptors developed by the international potato center (CIP). Qualitative data including plant type (PT), leaf shape (LSH), and lobes type (TL) was observed at vegetative growth stage (60 DAP) from each putative mutant lines and control variety (Table S2).

Quantitative traits associated with growth and development was also evaluated. Days to sprouting (SD) was recorded from planting until the first shoot emerged above the soil surface. Days to flowering (FD) was measured after planting once the first flower opened for each plant. Vine length (VL) was measured from the base of the plant to the tip of the longest vine using a standard ruler at vegetative growth stage. Leaf number per plant (LN) and branch number per plant (BN) were counted manually from each plant at vegetative growth stage (60 DAP).

Photosynthetic pigments, Chlorophyll a (Chla), chlorophyll b (Chlb), total chlorophyll (TC), carotenoids (CAR) of **t**he leaf tissue were examined by referring to Wintermans & De Mots [25]. Leaf samples were collected from the second and third nodes below the shoot tip from each treatment. A 0.2 g fresh leaf sample

was placed in a 15-mL beaker vial with 10 mL of 95% ethanol, and the vial sealed with Parafilm. The supernatants were detached after the regulated sample mix was centrifuged at 10,000 rpm for 15 min at 4°c. An aliquot (0.5 mL) of the resulting extract was used for spectrophotometric determination of Chla, Chlb, and carotenoids at wavelength of 665 nm, 649 nm, and 470 nm, respectively using a UV-2450 spectrophotometer. The following formulas were used to calculate the concentration of chlorophyll and carotenoids:

$$Chlorophyll\ a\ \left(\frac{mg}{g}\right) = \frac{\{(13.7\ x\ A665) - (5.76\ x\ A649)\}}{10}$$

$$Chlorophyll\ b\ \left(\frac{mg}{g}\right) = \frac{\{(25.8\ x\ A649) - (7.60\ x\ A665)\}}{10}$$

$$Total\ chlorophyll\ \left(\frac{mg}{g}\right) = \frac{\{(6.10\ x\ A665) + (20.0\ x\ A649)\}}{10}$$

$$C\ x + c\ \left(\frac{mg}{g}\right) = \frac{1000A470 - 2.13Ca - 97.63Cb}{209}$$

Chlorophyll fluorescence (Fv/Fm)

Chlorophyll fluorescence, an indicator of photosystem II efficiency, data was collected from the adaxial surface of the youngest fully expanded leaf of each plant. Handy-*PEA* fluorimeter (Hansatech, Kings Lynn, UK) was used to measure, using the methodology of Strasser et al. [26]. The leaf was dark adapted by leaf clip for 30 minutes before measurement. Light was then provided by an array of three high-intensity light-emitting diodes and adjusted to 1500 µmol m^{-2} s^{-1} to ensure that the photosynthesis was saturated. The measurement were taken at 60 days after planting (DAP).

2.5. Data Analysis

LD_{50} estimation and dose response curves were obtained using binomial logistic regression. Graphical visualization were performed in R software using the ggplot2, dplyr, MASS, and cowplot packages.

Descriptive statistics, including mean, range, standard deviation (SD), and coefficient of variation (CV) were calculated for the quantitative morpho-physiological traits to summarize the magnitude and dispersion of the phenotypic variation. For each vegetative generation, the quantitative trait data were analyzed according to the augmented randomized complete block design (augmented RCBD), with the three control varieties (Alamura, Kabode, and Awassa-83) treated as replicated checks and the putative mutant entries as unreplicated test entries. Analysis of variance and least significant difference mean comparison were performed using the

augmentedRCBD package in R, with statistical significance assessed at p = 0.05. The three control varieties were specified as checks in the analysis.

Genotypic and phenotypic coefficient of variation (GCV and PCV), genetic advance (GA), genetic advance as a percentage of the mean (GAM), and H^2 were estimated using the gva.augmentedRCBD() function from the augmentedRCBD package in R. [27], suggestion were used to classify PCV, GCV, and GAM as low (<10%), moderate (10–20%), and high (>20%). Broad-sense heritability (H^2) was categorized as low (<30%), moderate (30-60%), and high (>60%) according to Johnson et al. [28], Pearson correlation analysis was used to determine the linear relationship among the morpho-physiological traits using the corrplot package in R [29].

Principal Component Analysis (PCA) was used to explain multivariate phenotypic variation and identify traits contributing to the observed variation. Hierarchical clustering analysis (HCA) was subsequently used to group entries according to similarities in their multivariate trait profiles. PCA and HCA were performed using the FactoMineR package [30]. The resulting PCA and HCA classifications were used to identify clusters with desirable overall phenotypic performance and guide the advancement of putative mutant entries from M1V1 to M1V2 and from M1V2 to M1V3. R software version 4.5 was used for all analyses.

3. RESULT

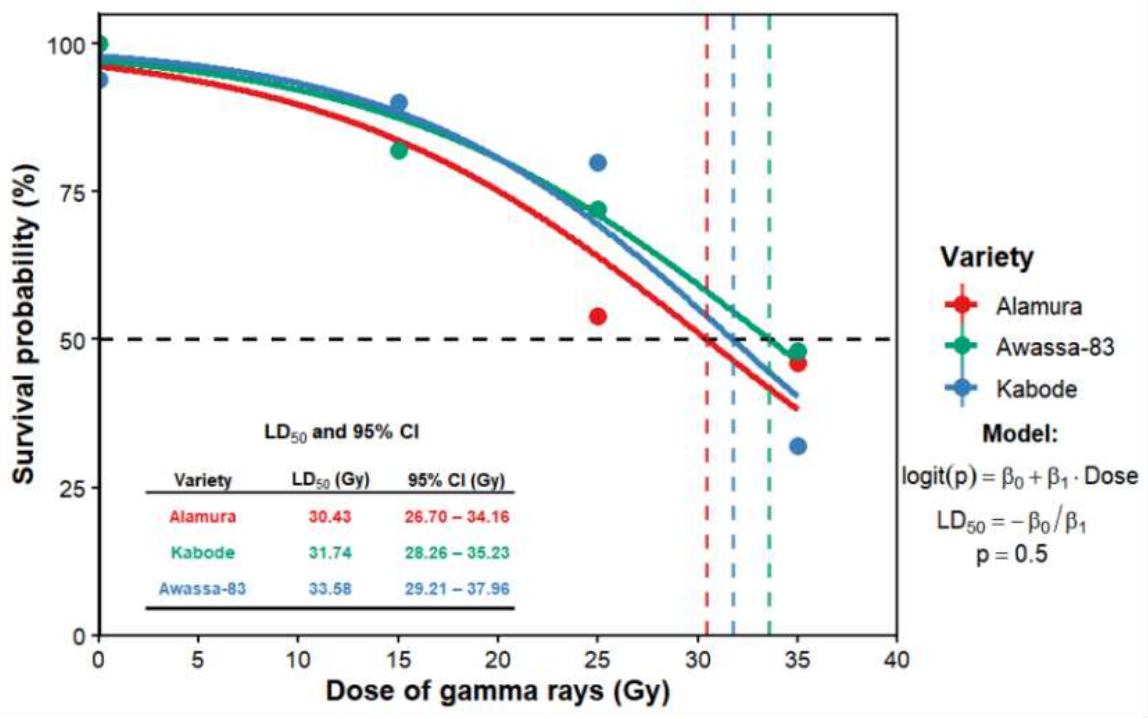
3.1. Radio sensitivity test: Determination of the LD50 of mutagens

The result indicated a clear decline in both sprouting and survival percentage as the dose of gamma irradiation increases (Table 2). Specifically, the percent sprouting was decreased from 86, 94, and 90 at 15 Gy to 56, 36, and 64% at 35 Gy for the varieties Alamura, Kabode, and Awassa-83, respectively. Similarly, survival percentage declined from 82, 90, and 82% at 15 Gy to 46, 32, and 48% at 35 Gy for varieties Alamura, Kabode, and Awassa-83, respectively (Table 2). The maximum percentage reduction in both sprouting and survival compared to the control was observed at 35 Gy, followed by 25 Gy. This trend is consistent across all varieties, demonstrating that higher doses of gamma irradiation lead to reduced sprouting and survival, thereby reducing establishment capacity over control. The estimated LD_{50} values with 95% confidence interval (CI) were 30.43 Gy (26.70 - 34.16) for Alamura, 31.74 Gy (28.26 - 35.23) for Kabode, and 33.58 Gy (29.21 - 37.96) for Awassa-83 (Fig. 1). Awassa-83 exhibited the highest LD_{50} value and comparatively greater tolerance to gamma

irradiation, whereas Alamura showed the lowest LD_{50} and showed the greatest sensitivity to gamma irradiation. Kabode displayed intermediate response. This result indicates that the three varieties respond differently to gamma irradiation. The estimated LD_{50} values provided a basis for evaluating the survival response of the irradiated lines.

Table 2. Effect of gamma irradiation on sprouting and survival percentage of different sweetpotato varieties

Variety	Gamma ray dose	Number of cutting	Percent sprouting	Percent Reduction over control	Percent Survival	Percent Reduction over control
Alamura	Control	50	100	-	100	
	15 Gy	50	86	14	82	18
	25 Gy	50	60	40	54	46
	35 Gy	50	56	44	46	54
Kabode	Control	50	96	-	94	2
	15 Gy	50	94	2	90	4
	25 Gy	50	88	8	80	14
	35 Gy	50	36	60	32	62
Awassa-83	Control	50	100		100	
	15 Gy	50	90	10	82	18
	25 Gy	50	76	24	72	28
	35 Gy	50	64	36	48	52



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295 **Figure 1**. Dose-response relationship between gamma irradiation dose and survival probability of sweetpotato
296 varieties. Points represent observed survival, and lines represent fitted binomial logistic regression models. The
297 horizontal dashed line indicates 50% survival, while vertical dashed lines indicate the estimated LD_{50} for each
298 variety. LD_{50} estimates and their 95% confidence intervals are shown in the inset table.

299 **3.2. Qualitative Phenotypic Variation**

300 The three qualitative traits, plant type (PT), leaf shape (LSH), and lobe type (TL), showed variation among the
301 evaluated entries across the three successive vegetative generations (Table 3). The Shannon-weaver diversity
302 index (H') indicated moderate to high diversity for qualitative traits, with the lope showing the highest diversity
303 index in all vegetative generations. The H' value for lobe type consistently increased from 1.5782 in M1V1 to
304 1.6008 in M1V2, and 1.631 in M1V3 vegetative generations, indicates considerable variation in the distribution
305 of lobe type categories among the evaluated entries (Table 3, Fig. 2).

306 Plant type showed a progressive change in the distribution of categories across successive vegetative
307 generations. In M1V1, semi-erect plants were the predominant category (53%), followed by spreading (28%),
308 erect (12%), and extremely spreading plants (7%). Both in M1V2 and M1V3, spreading plants constituted the
309 largest proportion, followed by extremely spreading, semi-erect, and erect plants (Table 3). These changes
310 demonstrate shifts in the observed phenotypic distribution of plant architecture across successive vegetative
311 generations.

Overall, the qualitative traits demonstrated substantial phenotypic diversity among evaluated entries. The observed changes in category frequencies across generations indicate that phenotypic distributions changed during successive vegetative generations, providing useful variation for subsequent multivariate characterization and putative mutant selection.

Table 3. Diversity of qualitative traits as indicated by Shannon-Weaver index (H') for sweetpotato putative mutant lines and their control varieties across successive vegetative generations

Trait	Score code (Phenotypic classes)	Number of classes	Shannon weaver index (H')	Evenness
	M1V1			
Plant type (PT)	Erect (12%); semi-erect (53%); spreading (28%); extremely spreading (7%)	4	1.1327	0.8173
Leaf shape (LSH)	Triangular (45%); hastate (18%); lobed (37%)	3	1.0354	0.9412
Lobe type (TL)	No lateral lobes (2%); very slight (8%); slight (29%); moderate (29%); deep (19%); very deep (13%)	6	1.5782	0.8806
	M1V2			
Plant type (PT)	Erect (5%); Semi-erect (23%); Spreading (49%); Extremely spreading (23%)	4	1.1754	0.8483
Leaf shape (LSH)	Triangular (49%); Hastate (16%); and Lobed (35%)	3	1.0103	0.9197
Lobe type (TL)	No lateral lobes (2%); Very slight (12%); Slight (31%); Moderate (24%); Deep (20%); Very deep (11%)	6	1.6008	0.8945
	M1V3			
Plant type (PT)	Erect (2%); Semi-erect (10%); Spreading (64%); Extremely spreading (24%)	4	0.9600	0.6920
Leaf shape (LSH)	Triangular (53%); Hastate (15%); Lobed (32%)	3	0.9860	0.8970
Lobe type (TL)	No lateral lobes (4%); Very slight (12%); Slight (33%); Moderate (24%); Deep (18%); Very deep (10%)	6	1.6310	0.9090

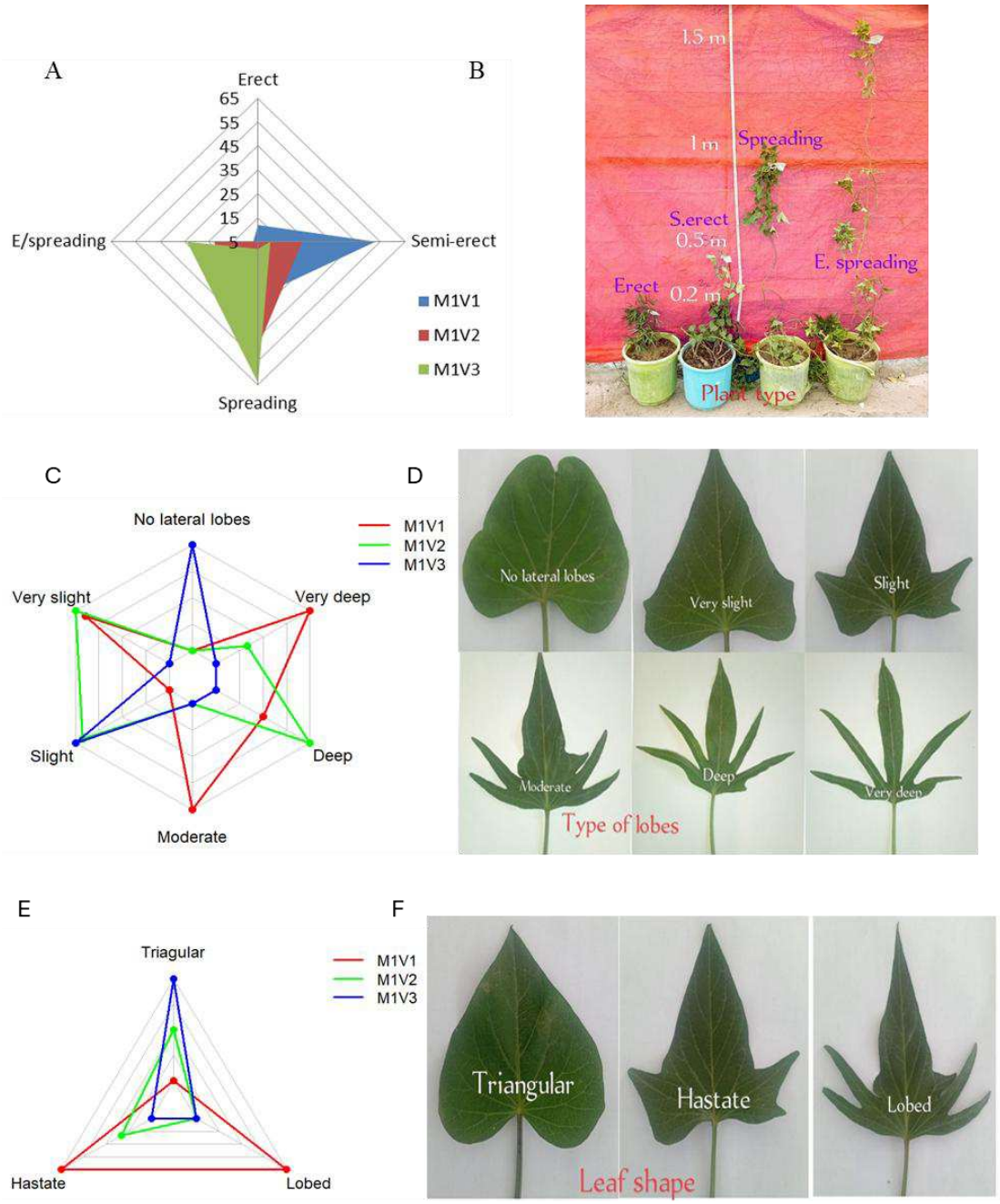

Figure 2. Radar chart and images across generations presenting the distribution and frequency of qualitative traits such as plant type (A, B), lobe types (C, D) and leaf shape (E, F)

3.3. Descriptive statistics of quantitative traits

Significant variation was exhibited among the evaluated entries (296, 257, and 178 for M1V1, M1V2, and M1V3, respectively) for quantitative traits across the three successive vegetative generations (Table 4). The magnitude of variation differed among traits and generations, as reflected by the coefficients of variation (CV), and ranges.

Among the quantitative characters, carotenoids exhibited the highest coefficient of variation (26.44%) for M1V1. In M1V2, branch number showed the highest variation, with a CV of 23.16%, whereas in M1V3 its CV decreased to 19.58%. In contrast, the lowest CV was observed from days to sprouting (4.25%) for M1V1, and days to flowering (5.83%) for M1V2, and leaf number (1.75%) for M1V3. Vine length showed the widest range of phenotypic values across all vegetative generations ranging from 5-230, 21-248cm, and 23-248cm for the M1V1, M1V2 and M1V3 respectively, while the narrowest ranges were observed for CAR (1.10-9.04) in M1V1, and chlorophyll fluorescence in M1V2 (0.27-0.99) and M1V3 (0.2-0.88) (Table 4).

Overall, the finding exhibit that considerable quantitative phenotypic variation was present among the evaluated entries and persisted across successive vegetative generations. Differences in magnitude of variation among traits suggest that the trait contributed unequally to the phenotypic differentiation of the populations. This variation provided the basis for subsequent estimation of genetic parameters and multivariate analyses used to characterize the putative mutant lines (Refer Appendix Table 1, 2, and 3 for ANOVA).

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Table 4: Estimation of variation in quantitative traits for sweetpotato putative mutant lines in M1V1, M1V2, and M1V3 vegetative generations

	M1V1				M1V2				M1V3			
Traits	Mean	CV (%)	Range	Sig	Mean	CV (%)	Range	Sig	Mean	CV (%)	Range	Sig
SD	16.3±0.7	4.25	7-31	***	-	-	-	-	-	-	-	-
FD	95.9±4.4	4.60	69-124	***	96.9±1.1	5.83	73-129	***	88.77±2.3	2.59	45-123	***
BN	4.9±0.69	14.0	1-15	***	5.00±1.16	23.16	1-14	*	5.19±1.02	19.58	1-14	*
LN	42.1±2	4.7	5-134	***	61.92±7.3	11.79	22-152	***	63.16±1.12	1.75	20-115	***
VL	49.4±2.2	4.5	5-230	**	76.79±12.4	16.10	21-248	***	75.1±4.63	6.17	23-248	***
Chla	6.7±0.9	12.8	2.05-18.4	***	7.64±0.83	10.84	2.83-5.8	**	7.67±0.73	9.56	1.24-13.30	**
Chlb	4.9±0.89	18.5	1.2–14.1	***	6.77±1.11	16.33	2.9-14.8	***	6.47±0.41	6.41	2.31-13.17	***
TC	11.5±1.7	14.6	5.67-32.5	**	14.39±1.40	9.75	8.5-30.65	***	14.17±0.79	5.58	2.38-23.41	***
CAR	3.52±0.93	26.44	1.10-9.04	*	2.65±0.59	22.47	0.68-8.72	***	3.21±0.48	14.91	1.06-6.39	***
Fv/Fm	-	-	-	-	0.67±0.08	12.64	0.27-0.99	***	0.64±0.04	5.46	0.2-0.88	***
TN	-	-	-	-	-	-	-	-	4.39±0.34	7.94	1-8	***

*Note: *, **, *** indicate significant at p<0.0 5, p<0.01, and p<0.001, respectively. PT=plant type, LSH=leaf shape, TL=lobe types, SD=days to sprouting, FD=days to flowering, BN= branch number, LN=leaf number, VL= vine length, Chla=chlorophyll a, Chlb= chlorophyll b, TC=total chlorophyll, CAR=carotenoids, Fv/Fm= chlorophyll fluorescence, TN= tuber number*

375 **Table 5**. Estimates of genotypic variance (GV), phenotypic variance (PV), genotypic (GCV) and phenotypic (PCV) coefficients of variation, genetic advance (GA),
376 genetic advance as a percentage of the mean (GAM) and broad-sense heritability (H^2) for quantitative traits of *sweetpotato putative mutant lines.M1V1, M1V2, and M1V3*

Generations	**Quantitative traits**											
		SD	FD	BN	LN	VL	Chla	Chlb	TC	CAR	Fv/Fm	TN
M1V1	PV	38.84	133.93	4.75	351.60	858.86	3.24	3.98	10.51	2.07	-	-
	GV	38.37	114.57	4.27	347.68	853.86	2.50	3.17	7.68	1.20	-	-
	GCV	36.50	11.12	42.26	45.04	62.59	23.53	36.75	24.00	31.55	-	-
	PCV	36.73	12.03	44.56	45.30	62.77	26.79	41.14	28.06	41.38	-	-
	GA	12.70	20.42	4.04	38.25	60.11	2.87	3.28	4.89	1.73	-	-
	GAM	74.84	21.23	82.68	92.41	128.74	42.64	67.72	42.33	49.63	-	-
	H^2	98.77	85.55	89.94	98.89	99.42	77.15	79.79	73.11	58.13	-	-
M1V2	PV	-	300.35	3.45	396.95	1264.4	2.90	3.94	9.07	1.86	0.03	-
	GV	-	268.4	2.10	343.65	1111.5	2.21	2.72	7.10	1.51	0.03	-
	GCV	-	16.78	29.16	30.44	45.97	19.27	23.92	18.25	45.99	24.89	-
	PCV	-	17.75	37.32	32.72	49.03	22.06	28.80	20.63	51.12	27.97	-
	GA	-	31.96	2.34	35.58	64.48	2.68	2.82	4.86	2.28	0.30	-
	GAM	-	32.72	46.99	58.44	88.92	34.74	40.97	33.31	85.05	45.68	-
	H^2	-	89.38	61.03	86.57	87.90	76.35	68.97	78.27	80.94	79.16	-
M1V3	PV	-	309.4	3.66	265.9	888.5	3.89	3.88	11.41	2.07	0.03	3.76
	GV	-	304.08	3.44	264.68	867	3.35	3.71	10.79	1.84	0.03	3.64
	GCV	-	19.61	35.51	26.27	41.73	24.11	29.90	23.34	43.30	26.42	45.02
	PCV	-	19.78	36.6	26.33	42.24	25.97	30.59	24.01	45.93	26.99	45.8
	GA	-	35.66	3.7	33.49	60.04	3.51	3.89	6.59	2.64	0.34	3.9
	GAM	-	40.10	71.0	54.07	85.04	46.16	60.30	46.81	84.20	53.34	91.4
	H^2	-	98.28	93.93	99.54	97.6	86.15	95.56	94.52	88.85	95.79	96.75

377 *Note: SD=days to sprouting, FD=days to flowering, BN= branch number, LN=leaf number, VL= vine length, Chla=chlorophyll a, Chlb= chlorophyll b, TC=total*
378 *chlorophyll, CAR=carotenoids, Fv/Fm= chlorophyll fluorescence, TN= tuber number*

3.4. Variance components and heritability

Phenotypic variance (PV) ranged from 2.07 to 858.86, 0.03 to 1264.4, and 0.03 to 888.5 for first vegetative generation (M1V1), second vegetative generation (M1V2), and third vegetative generation (M1V3), respectively. Regarding genotypic variance (GV), VL has the highest estimated values of 853.86, 1111.5, and 867 for M1V1, M1V2, and M1V3 generations, respectively. The lowest is CAR (1.20) for M1V1, Fv/Fm 0.03 for M1V2, and 0.03 for M1V3 (Table 5).

In M1V1, genotypic coefficients of variation (GCV) values were found to be high for VL (62.59%), LN (45.04%), BN (42.26%), Chlb (36.75%), SD (36.5%), CAR (31.55%), TC (24%), and Chla (23.53%), whereas FD (11.12%) had moderate GCV values. In M1V2, GCV values were found to be high for VL (45.97%), CAR (45.99%), LN (30.44%), BN (29.16%), Fv/Fm (24.89%), and Chlb (23.92%), while moderate GCV values were obtained for Chla (19.27%), TC (18.25%), and FD (16.78%). In M1V3, except FD (19.61%) scores moderate GCV values, the other traits score high GCV values TN (45.02%), CAR (43.30%), VL (41.73%), BN (35.51%), Chlb (29.90%), Fv/Fm (26.42%), LN (26.27%), Chla (24.11%), and TC (23.34%) (Table 5).

PCV values in M1V1 were found to be high for VL (62.77%), LN (45.30%), BN (44.56%), CAR (41.38%), Chlb (41.14%), SD (36.73%), TC (28.06%), Chla (26.79%), and moderate PCV was scored for FD (12.03%). In the second generation, all traits except FD (17.75%) had high PCV values which are greater than 20%. In M1V3, all the traits had high PCV values except FD (19.78), which scored a moderate PCV value (Table 5).

GCV and PCV for M1V1 ranged from 11.12% to 62.59% and 12.03% to 62.77%, respectively. While in M1V2, GCV and PCV ranged from 16.78% to 45.99% and 17.75% to 51.12%, respectively. For M1V3, GCV and PCV ranged from 19.61% to 45.02% and 19.78% to 45.93%, respectively (Table 5).

Genetic advances as a percentage of the mean (GAM) ranged from 21.23% to 128.74% for M1V1, 32.72% to 88.92% for M1V2, and 46.16% to 91.4% for M1V3. For M1V1, we found a high genetic advance as a percentage of the mean for vine length (128.74%), LN (92.41%), BN (82.68%), CAR (49.63%), Chla (42.64), Chlb (67.72%), and TC (42.33%). In M1V2, all traits scored a high genetic advance as a percentage of the mean. In M1V3, GAM was obtained for all traits (Table 5).

Broad-sense heritability is the proportion of phenotypic variance attributed to genetic variance under the conditions of the experiment. Heritability in the broad sense ranged from 58.13% to 99.42%, 61.03% to 89.38%, and 86.15% to 99.54% for M1V1, M1V2, and M1V3 respectively. In M1V1, most of the studied traits showed

high heritability; while moderate heritability exhibited from CAR (58.13%). In M1V2 and M1V3, all of the studied traits showed high heritability (Table 5).

Across the three vegetative generations, the variability parameters demonstrated that the putative mutant populations retained considerable variation for several quantitative traits. This estimate provided a quantitative basis for identifying traits with greater potential response to selection and complemented the subsequent correlation, principal component, and cluster analysis.

3.5. Inter-trait Correlation

Pearson correlation analysis exhibited varying degrees of association among the quantitative traits across the three vegetative generations. The strongest positive and significant correlation (r = 0.87, 0.86, and 0.85) was obtained between Chlb and TC with correlation coefficient of 0.87, 0.86, and 0.85 in M1V1, M1V2, and M1V3, respectively. Chla, CAR, and Fv/Fm were positive and significantly correlated with tuber number in the third generation. The association between FD and VL was weak and negative in all vegetative generations, with a correlation coefficient of -0.06, -0.23, and -0.11 for M1V1, M1V2, and M1V3, respectively (Fig. 3).

Overall, the correlation analysis demonstrated that the strength and significance of trait associations varied across generations. The consistent strong correlation between Chlb and TC, together with the associations involving photosynthetic traits and tuber number in M1V3, identified traits that contributed to the phenotypic relationships within the putative mutant lines.

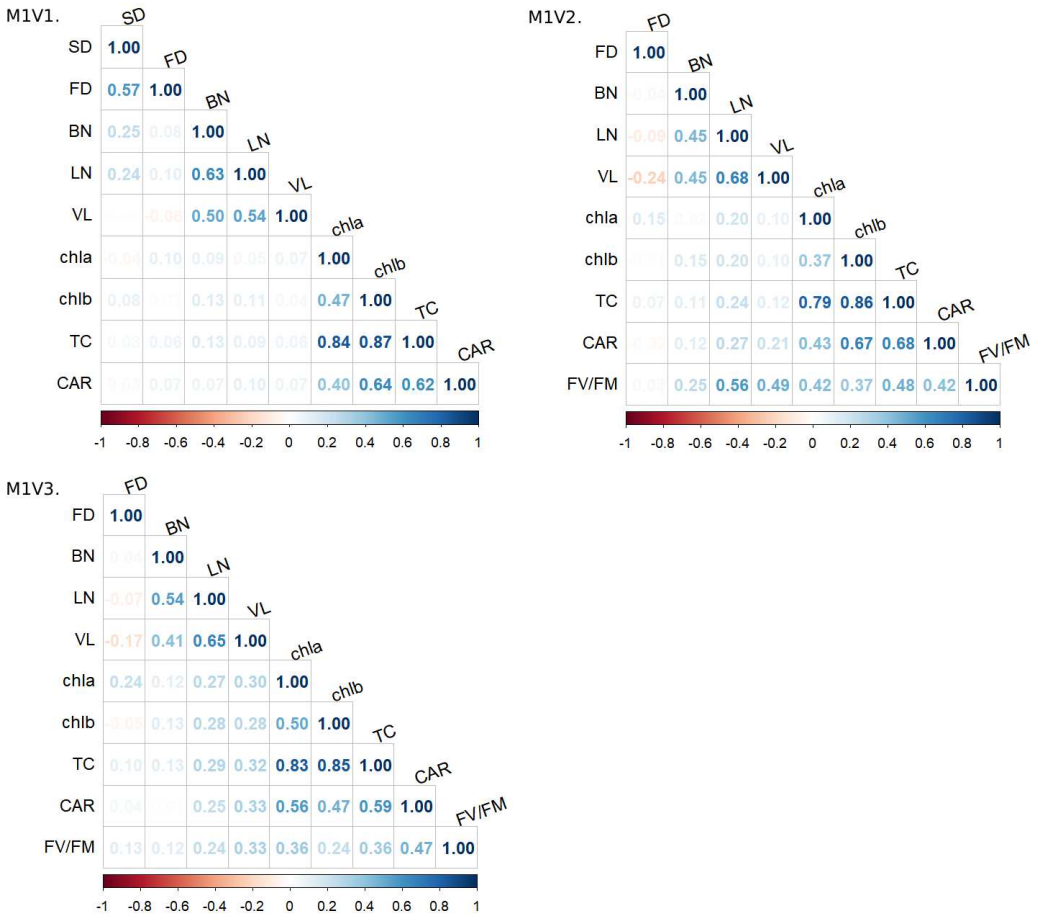

Figure 3. Coefficient of correlations among the quantitative traits of 296, 257, and 178 entries for M1V1, M1V2, and M1V3 vegetative generations

3.5. Principal Component Analysis (PCA)

The first four principal components were retained because they collectively explained 82.26%, 82.19%, and 80.18% of the total variation in M1V1, M1V2, and M1V3, respectively (Fig. 4, Table 6).

In M1V1, PC1, PC2, PC3, and PC4 with eigenvalues of 3.07, 2.11, 1.52, and 0.70 contributed to 34.10%, 23.49%, 16.88%, 7.78%, respectively, and together explaining 82.26% (Fig. 4, Table 6). In PC1, the strong contributors were total chlorophyll (TC) and chlorophyll b (Chlb). In PC2, the branch number (BN) and leaf number (LN) had considerable influence. Days to flowering (FD) and days to sprouting (SD) contributed to PC3, whereas chlorophyll a (Chla) and carotenoids (CAR) contributed to PC4 (Table 6)

In M1V2, PC1, PC2, PC3, and PC4 with eigenvalues of 3.65, 1.93, 1.04 and 0.78 contributed to 40.62%, 21.48%, 11.5%, and 8.63 of total variation, giving cumulative contribution 82.19% (Fig. 4, Table 6). TC, Chlb, CAR, and Fv/Fm contributed to PC1; LN and VL for PC2; FD for PC3 and BN for PC4 (Table 6)

In M1V3, PC1, PC2, PC3, and PC4 had eigenvalues of 4.19, 1.68, 1.15, and 1.01, respectively, and contributed to 41.85%, 16.75%, 11.51%, and 10.07%, respectively, and together explaining 80.185% of the total variation (Fig. 4; Table 6). In PC1, the strong contributors were TC, Chla, TN, and CAR. In PC2, the important contributors were BN, LN, and VL, whereas FD, Chlb, and Fv/Fm contributed to PC3 (Table 6).

The PCA results demonstrate that different groups of morpho-physiological traits contributed to the differentiation of the evaluated entries in each generation. The relatively high cumulative variation explained by the first four components indicates that a substantial proportion of the multivariate variation could be summarized using these components. These results provided a basis for subsequent multivariate grouping and putative mutant selection.

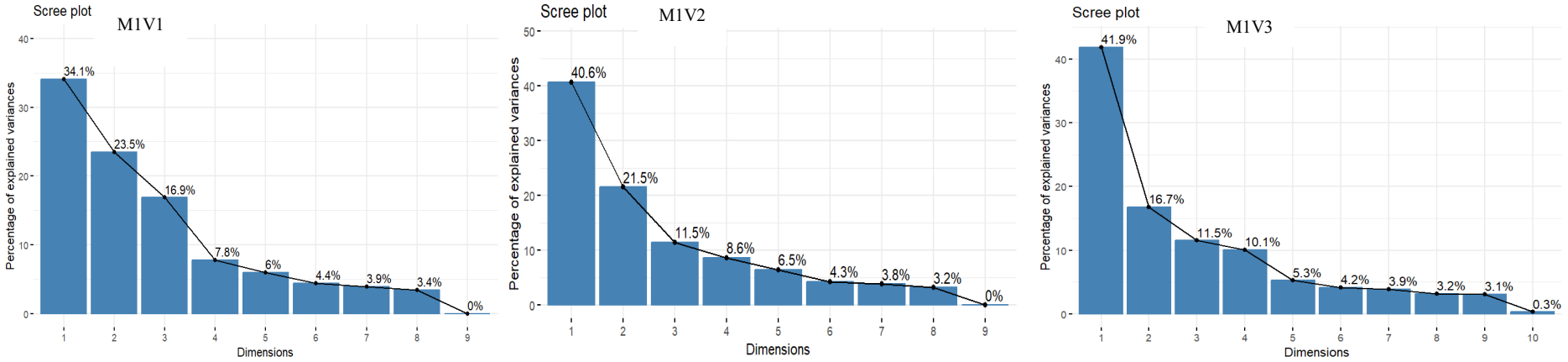

Figure 4. Scree plot of quantitative variables represented on the principal components for 296, 257, and 178 sweetpotato entries for M1V1, M1V2, and M1V3.

Table 6: PCA and Eigenvalues of the morpho-physiological traits of sweetpotato entries evaluated in M1V1, M1V2, and M1V3 vegetative generations

Variables	**M1V1**					**M1V2**					**M1V3**				
	PC1	**PC2**	**PC3**	**PC4**	**PC5**	**PC1**	**PC2**	**PC3**	**PC4**	**PC5**	**PC1**	**PC2**	**PC3**	**PC4**	**PC5**
Eigenvalues	3.07	2.11	1.52	0.70	0.54	3.65	1.93	1.03	0.78	0.58	4.19	1.68	1.15	1.01	0.53
%Variance	34.102	23.49	16.88	7.78	5.96	40.62	21.48	11.5	8.63	6.46	41.85	16.75	11.51	10.07	5.25
% Cumulative	34.102	57.59	74.47	82.26	88.22	40.62	62.12	73.56	82.19	88.66	41.85	58.6	70.10	80.18	85.43
Eigen vectors															
SD	0.99	9.42	37.47	2.45	5.52	-	-	-	-	-	-	-	-	-	-
FD	0.84	3.24	45.01	4.93	17.32	0.00	6.16	75.01	5.31	8.11	0.24	7.82	36.33	35.75	3.00
BN	4.50	26.44	2.09	0.02	17.83	3.97	15.98	2.17	46.84	22.04	2.70	27.90	1.71	16.88	21.90
LN	4.04	28.58	1.96	0.70	0.56	10.25	19.59	2.03	1.55	1.82	7.79	26.01	0.00	1.44	6.40
VL	2.41	19.61	13.44	1.90	32.34	7.13	26.91	0.04	2.74	0.66	9.02	20.12	0.05	2.23	17.11
Chla	18.34	3.24	0.02	46.35	0.06	12.21	7.82	4.18	15.87	37.34	14.71	5.78	0.15	5.83	8.67
Chlb	23.15	3.00	0.00	12.25	4.31	15.52	7.04	6.68	13.48	4.22	12.07	2.25	21.90	2.36	14.61
TC	28.31	4.24	0.00	2.73	1.98	20.29	10.75	0.31	0.04	4.13	17.15	5.58	9.19	4.88	0.73
CAR	17.42	2.23	0.00	28.68	20.08	15.52	3.33	5.17	2.80	14.22	13.17	3.98	0.00	7.06	7.66
Fv/Fm	-	-	-	-	-	15.11	2.41	4.40	11.37	7.46	9.40	0.32	19.58	15.40	18.86
TN	-	-	-	-	-	-	-	-	-	-	13.76	0.24	11.08	8.18	1.08

***Note**: PC1, PC2 … indicates principal component analysis; SD (days to sprouting), FD (days to flowering), BN (branch number), LN (leaf number), VL (vine length (VL), Chla (chlorophyll a), Chlb (chlorophyll b), TC (total chlorophyll), CAR (carotenoids), Fv/Fm (chlorophyll fluorescence), and TN (tuber number). M1V1, M1V2, M1V3 (1st, 2nd and 3rd generations)*

3.6. PCA-Biplot

To assess trait variation among irradiated sweetpotato lines, the PCA biplot analysis related the eigenvalues of both Dim1 and Dim2, resulting from principal component analysis (PCA) were considered.

In M1V1, the total of 57.65% of the variation was accounted for by the first two principal components. The analysis identified that TC, Chlb, LN, and BN as strongly contributing traits for to the separation of entries along PC1 and PC2. Acute angles between vectors of morphological traits (VL, BN, LN, SD, and FD) and physiological traits (Chla, Chlb, TC, and CAR) indicated positive correlation, and the right angles between traits suggested no correlation. The putative mutant lines AL3540, AL356, AL355, AL353, AL251, and K251 positioned far from the biplot origin, indicating multivariate deviation compared to irradiated sweetpotato lines near the biplot origin (Fig. 5M1V1).

In M1V2, the first two principal components (Dim1 and Dim2) provided a comprehensive visualizing analysis of 62.1% of the total variability (Fig. 4M1V2). Positive correlations between morphological traits VL, LN, were revealed, while FD negatively correlated with VL, LN and BN. In addition, physiological traits TC, Fv/Fm, Chla, Chlb, and CAR were positioned in a similar direction, indicating positive correlation. TC, Fv/Fm, CAR, Chlb, VL, LN, and BN were strongly contributed traits for PC1 and PC2 (Fig. 4B). AL3540, AL356, AL355, AL353, and AL151 were positioned relatively far from the biplot origin, showing phenotypic variance than the rest of the putative mutant lines (Fig. 5M1V2).

In M1V3, the first two PCAs explained 57.9% of total variability. TC, Chla, LN and BN were the traits that contributed strongly to PC1 and PC2. The PCA biplot revealed a strong association between morphological variables such as BN, LN, and VL; as well as among physiological variables TC, Chla, Chlb, CAR, Fv/Fm. Conversely, FD has negative relation with VL and LN. AL3540, AL356, AL355, AL1545, and K1525 were most diverse from the biplot origin reflecting more variance compared to the rest of irradiated sweetpotato lines. AL2520 was located in the biplot origin and reflecting the least variability under irradiated lines (Fig. 5M1V3).

Across the three vegetative generations, the first two PCA (Dim 1and 2) explained 57.5, 62.2, and 57.9% of total variability, respectively. The morphological traits showed positive associations with each other; there is also a positive association between physiological traits. FD shows negative correlation with VL and LN telling trade-off between vigorous vegetative developments. TC, Chlb, LN, and BN repeatedly emerged as the most influential traits contributing to multivariate differentiation across generations. AL3540, AL356, AL355

consistently appeared far from the biplot origin in all generation, indicating consistent multivariate divergence across generations.

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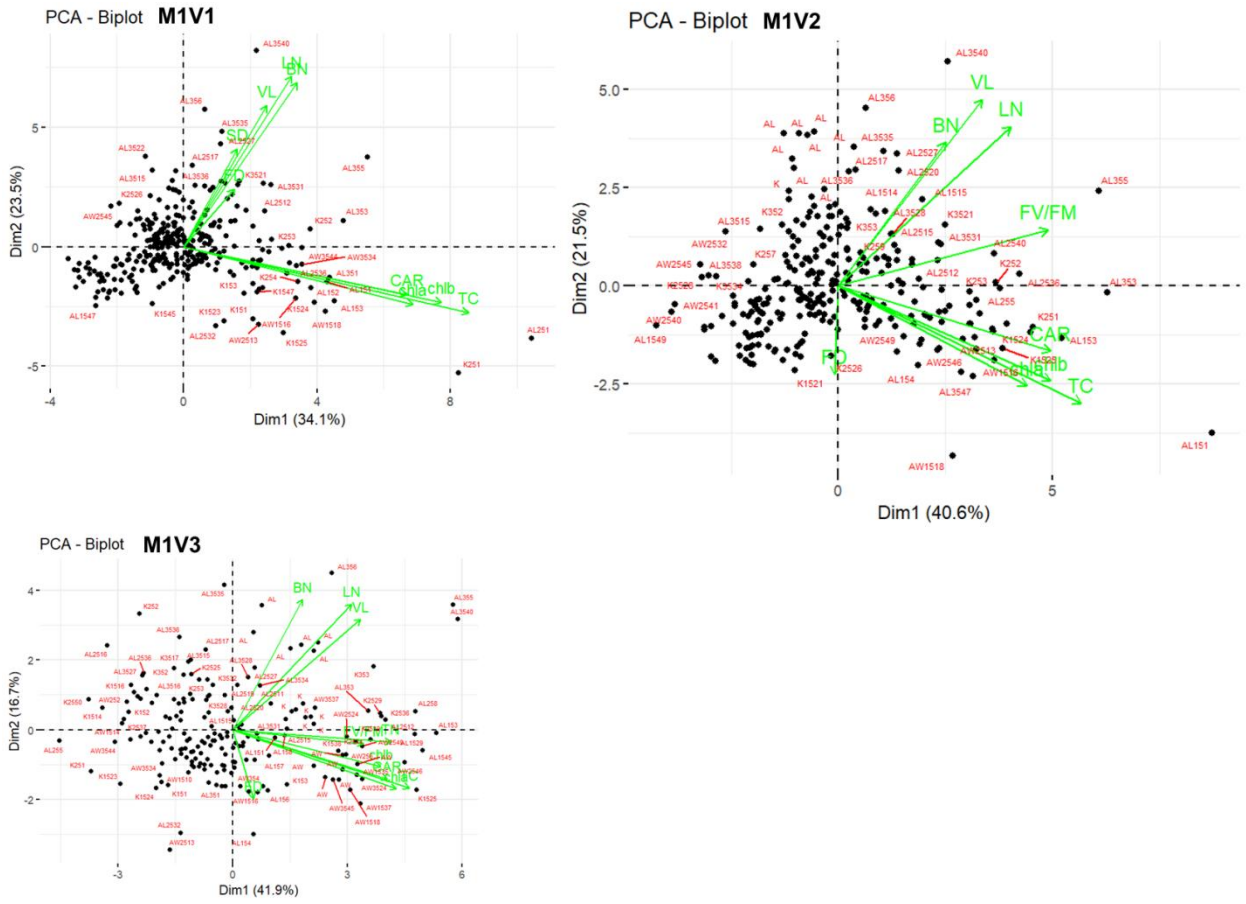

Figure 5: Biplot of quantitative variables represented on the principal components for 296, 257, and 178 Sweetpotato entries for M1V1, M1V2, and M1V3 generations. SD=days to sprouting, FD=days to flowering, BN=branch number, VL=vine length, LN=leaf number, Chla= chlorophyll a, Chlb= chlorophyll b, TC=total chlorophyll, CAR= carotenoids, Fv/Fm= chlorophyll fluorescence, TN= tuber number

3.7. Cluster analysis

In the first generation (M1V1), out of 9 quantitative traits considered, PCA helped to identify eight traits (SD, FD, BN, LN, Chla, Chlb, TC, and CAR) that were highly distinguishable and useful for sweetpotato putative mutant lines characterization. Using these eight quantitative variables and the UPGMA clustering method, the irradiated lines were classified into 5 clusters, namely C1, C2, C3, C4, and C5, which consisted of 31, 59, 132, 32, and 39 irradiated lines, respectively (Table 7). Cluster C1, characterized by high mean values of Chla, Chlb, TC, and CAR traits, while the lowest mean values were observed from cluster C5. In this generation, 39

irradiated lines belonging to cluster C5 were not selected for the next generation. Clusters with consistently lower mean values for greater number of the evaluated traits, relative to the other clusters, were excluded from further generation advancement.

In M1V2, 3 control and 254 sweetpotato putative mutant lines were categorized into 4 groups depending on their character similarity depending on eight quantitative traits (FD, BN, LN, VL, Chlb, TC, CAR, and Fv/Fm). The four clusters contained 46, 67, 79, and 62 for C1, C2, C3, and C4, respectively (Table 7, Fig. S.5). C3 was excluded based on their lower overall cluster means for the predefined morpho-physiological selection criteria, while 175 putative mutant lines and the 3 control entries belonging to C1, C2, and C4 were advanced to the next generation.

In M1V3, 3 control and 175 lines were categorized into 4 groups using 10 quantitative traits FD, BN, LN, VL, TN, Chla, Chlb, TC, CAR, and Fv/Fm. The cluster consisted of 84, 25, 20, and 46 lines for C1, C2, C3, and C4, respectively (Table 7). Cluster C1, characterized by high cluster mean values of all traits, while the Cluster C3 characterized by 6 traits with the lowest cluster mean values (Table 7). On the figure from M1V3 all the irradiated lines and control plants were selected to study the next generation. To see the individuals with their cluster over generations refer (Figure S1).

Overall, cluster analysis demonstrated that the phenotypic structure of putative mutant populations differed among generations. The exclusion of C5 in M1V1 and C3 in M1V2 reduced the number of entries were retained for subsequent phenotypic evaluation, while the remaining entries were retained foe subsequent phenotypic evaluation.

Table 7. Result of cluster analysis and cluster means of 296, 257, and 178 sweetpotato entries evaluated for M1V1, M1V2, and M1V3 generations

Generation	Cluster	SD	FD	BN	LN	VL	TN	Chla	Chlb	TC	CAR	Fv/Fm
M1V1 (296)	C1=31	14.9	93.6	5.06	34.7	-	-	9.87	8.18	18.0	5.86	-
	C2=59	22.5	113.	4.46	37.9	-	-	6.59	4.66	11.2	3.30	-
	C3=132 +3	13.3	91.3	5.01	43.6	-	-	6.52	4.50	11.0	3.38	-
	C4=32	24.7	99.3	8.09	77.0	-	-	6.30	5.34	11.6	3.46	-
	C5=39	13	87.7	2.62	19.9	-	-	5.23	3.28	8.5	2.44	-
M1V2 (257)	C1=46+2	-	85.8	5.40	74.4	104.	-	-	5.22	12.2	1.79	0.720
	C2=67+1	-	92.6	5.12	72.0	94.9	-	-	9.0	18.0	4.24	0.809
	C3=79	-	94.9	4.03	39.7	38.6	-	-	6.0	12.9	1.93	0.435
	C4=62	-	114.	5.66	65.6	76.5	-	-	6.72	14.3	2.59	0.744
M1V3 (178)	C1=84+1	-	77.6	5.23	64.4	75.1	3.67	7.40	6.57	13.9	2.78	0.574
	C2=25+2	-	94.4	5.86	78.1	108.	7.30	10.1	8.37	18.5	5.19	0.823
	C3=20	-	80.9	4.55	54.6	58.8	3.7	4.04	3.74	8.22	2.78	0.625
	C4=46	-	110.	4.85	52.5	55.5	3.80	7.82	5.95	13.8	2.69	0.639

SD (days to sprouting), FD (days to flowering), BN (branch number), LN (leaf number), VL (vine length (VL), Chla (chlorophyll a), Chlb (chlorophyll b), TC (total chlorophyll), CAR (carotenoids), Fv/Fm (chlorophyll fluorescence), and TN (tuber number). M1V1, M1V2, M1V3 (1st, 2nd and 3rd generations)

Note:- the numbers written after the plus sign are number of control varieties

4. DISCUSSION

Gamma irradiation has long been recognized as one of the most effective physical mutagens for generating novel genetic variation, particularly in clonally propagated crops where conventional hybridization is constrained by complex reproductive biology. In sweetpotato, the highly heterozygous hexaploid genome, self- and cross- incompatibility, and predominant vegetative propagation greatly limit the efficiency of conventional breeding. Consequently, induced mutagenesis provides an attractive alternative for broadening the genetic base while preserving the desirable genetic background of elite cultivars [31, 32].

The sprouting and survival percentage declined with increased dosage of gamma irradiation (Table 2). Increasing gamma irradiation doses from 15 to 35 Gy progressively reduced the establishment capacity of vine cuttings in all three varieties, although the magnitude of the response differed among varieties. The estimated LD_{50} values of 30.43 GY for Alamura, 31.74 Gy for Kabode and 33.58 Gy for Awassa-83 indicate differences in Radiosensitivity among the tested varieties. Variety Awassa-83 was the least sensitive to gamma irradiation doses, whereas Kabode showed comparatively lower tolerance to gamma irradiation under the conditions of this experiment. Similar irradiation-associated variation within the same species also observed in other plant species such as gamma-ray induced variation in chickpea [33], Laelia autumnalis on protocorm development [34], and Jerusalem artichoke [12]. Similar dose dependent reductions in survival and establishment have been reported in sweetpotato and other vegetatively propagated crops (6, 12, 35).

The reduction in sprouting and survival at high irradiation doses may be associated with radiation-induced cellular damage and disruption of physiological process required for successful establishment. Gamma irradiation may generate reactive oxygen species that affect metabolic activity, leading to growth delay and reducing survival at higher doses [36]. Radiation-induced effects on growth-regulating process and photosynthesis structures may also contribute to reduced establishment at higher doses [37, 38]. However the present study evaluated survival and phenotypic responses rather than the underlying cellular or molecular mechanisms; therefore, these mechanisms should be considered possible explanations rather than direct findings of this study.

The estimated LD_{50} values obtained in this study fall within the range commonly reported for vegetatively propagated crops and indicate that the intermediate irradiation doses provide the most suitable balance between

mutation induction and plant survival. Lower doses may induce relatively few mutations, whereas excessively high doses may cause severe physiological damage, chromosomal abnormalities and reduce survival. Therefore, identifying genotype specific LD_{50} values represents an essential first step in mutation breeding because it maximizes the probability of generating useful mutations while maintaining sufficient surviving populations for effective selection [3].

Phenotypic characterization of the qualitative and quantitative traits, combined with multivariate analysis, revealed considerable phenotypic variation. Gamma irradiation had a considerable effect on the qualitative traits such as plant type, leaf shape, and type of lobe across the three vegetative generations (M1V1, M1V2, and M1V3). The mutant population showed significant variation in the qualitative traits measured; as such, high to moderate values of Shannon-Weaver indices indicate a reasonable level of phenotypic variation in the putative mutant population across the three vegetative generations. In general, the predominant qualitative trait variation was observed for the type of lobe with high H' and evenness across the three vegetative generations. With regards to each qualitative trait in each generation, plant types which are semi-erect in M1V1 - M1V2 and spreading and extremely spreading in M1V2 and M1V3; triangular-shaped leaves M1V1- M1V3 and moderately to slightly lobed types dominated in the population. This has been shown in previous findings that gamma irradiation altered qualitative traits of sweetpotato [35], and Mung bean crops [39]. Shin et al. [40], illustrated that sweetpotato exhibited great phenotypic variation due to its out-crossing nature, combined with vegetative propagation. This change in the phenotypic traits may be due to mutation like, single-base substitutions, deletions, insertions, inversions, or translocations occurring at the genome scale which caused by DNA failure to be repaired [41].

In a similar pattern, the measured quantitative morpho-physiological traits varied significantly among putative mutants across all generations. The magnitude of variation was high for some traits. such as carotenoids (26.44% for M1V1), branch number 23.16% for M1V2), and 19.58% (M1V3), showing genetic variability in the population which is crucial opportunity to improve multiple traits associated to plant vigor, photosynthesis performance and yield potential in breeding programs [42]. Similar high variations of quantitative traits were observed in other studies in cowpea crop [43]. Noticeably, the reduced number and delayed sprouting of the cuttings in M1V1 might be due to cell injury and damage caused by ionizing radiation, resulting in cell arrest [44]. Lower variation in days to sprouting and leaf number indicates stability, while a higher range in vine length suggests the ability for wider adaptation. These findings were similar to previous reports by [45], in

589 sugarcane and [46] in sweetpotato, indicating the mutation-induced variability as an important agent for
590 improving crop characters.

591 In the same manner, the analysis of phenotypic (PCV) and genotypic (GCV) variance across the three vegetative
592 generations exposes highly heritable traits among the putative mutants. The high GCV and PCV values
593 observed from the vine length and leaf number traits indicate the presence of phenotypic variability, providing
594 opportunities for effective selection and genetic improvement [47]. Moreover, the relatively small difference
595 between GCV and PCV suggest that environmental effects contributed relatively less to the observed variation
596 for these traits under the experimental conditions whereas; larger differences indicate greater environmental
597 contribution. Similar use of GCV and PCV for assessing variability in sweetpotato populations has been
598 reported by [48].

599 Broad-sense heritability was generally high across evaluated traits and generations. The high percentage of
600 heritability, especially in M1V2 and M1V3, implies that the character is highly influenced by genetic factors
601 rather than environmental factors under the experimental condition. However, heritability should not be
602 interpreted as proof of genetic stability nor as evidence that selection will necessary result in a large response.
603 The combined consideration of heritability, genetic advance, and genetic advance as percentage of the mean
604 provides a more useful indication of the potential effectiveness of selection [49].

605 The observed positive and significant correlation highlights the complicated relation between chlorophyll
606 contents in M1V1, M1V2, and M1V3 and chlorophyll contents with tuber number in M1V3. This result
607 recommends increased chlorophyll contents can improve photosynthesis capacity, which potentially leads to
608 greater tuber yields [50]. In contrast, the negative correlation between vine length and days to flowering
609 suggests a possible exchange in resource allocation, where a relatively earlier flowering may limit vegetative
610 growth [51]. The absence of the association between days to flowering and other traits in a population may be
611 affected by environmental, genetic factors or mutation itself [52]. The negative correlation between FD and
612 vegetative traits implies that breeders must balance flowering duration and vine growth for optimal yield [53].
613 Furthermore, PCA carried out across three generations of irradiated lines resulted in clear trends of the pattern
614 of variation in traits. Eigenvalues reflect a considerable contribution to the diversity in PCA, including M1V1,
615 where total chlorophyll and chlorophyll b played an important role in explaining diversity, while branch number
616 and leaf number emerged as influential in M1V2. The third generation also brought into light the importance of
617 chlorophyll a, total chlorophyll, carotenoids, and number of leaves, showing that within the generation, there

was an evolution of trait importance (Table 6). The first four principal components explained more than 80% of the total variation in each generation. comprising both morpho-physiological and agronomic traits including vine length, leaf number, chlorophyll content, Fv/Fm and tuber number, indicates structured, trait-driven variation rather than random dispersion (Table 6). The result was consistent with a previous study, which describes the importance of photosynthetic responses and growth characteristics in basil, highlighting the relevance of photosynthetic traits in evaluating plant responses to irradiation [54]. Breeding approaches for photosynthetic effectiveness and general plant vigor might benefit greatly from the progressive changes in the contribution of characteristics, which speak to the active nature of phenotypic variability in response to irradiation [55].

In addition, the PCA-biplot well took the morpho-physiological variability of sweetpotato putative mutant lines across three generations. The result showed key selective traits, trait correlations, and high-performing mutant lines, which are useful for sweetpotato breeding programs. The PCA exposed that the first two components accounted for approximately 58-62% of total variability, clearly differentiating the putative mutant lines based on their morpho-physiological characteristics. The small angle between leaf numbers, vine length, branch number showed strong association between vegetative growths. Chlorophyll pigments, carotenoids and chlorophyll fluorescence also created another close association presenting photosynthetic efficiency.

The heritability and the importance of traits such as TC, Chla, LN, and BN in gathering for photosynthetic efficiency, plant structure, and yield potential are recommended by their consistency over generations [7, 56]. The most diverse lines represented greater multivariate deviation from the overall centroid, but this position should not by itself be interpreted that superior agronomic performance.

Hierarchical cluster analysis further separated the putative mutant entries into distinct phenotypic groups. Clusters were evaluated based on their mean performance across the quantitative traits included in the cluster analysis. Clusters showing comparatively lower mean values for a greater number of traits were considered less promising and excluded from further generation advancement. In M1V1, five clusters were identified, and cluster 5, comprising 39 entries, were excluded from advancement based on the selection a criterion applies in the study. Consequently, 254 putative mutant entries were retained for M1V2. In M1V2, four clusters were identified, and Cluster 3, comprising 79 entries, was excluded resulting in 175 putative mutant entries were distributed among four clusters and were retained for further evaluation. The consistency between PCA, PCA-biplot, cluster analysis, and genetic parameter estimates further strengthen the reliability of these selections and

demonstrate the usefulness of multivariate approaches for identifying promising putative mutant lines in sweetpotato breeding programs.

Entries such as AL3540, AL356, and AL355 appeared repeatedly far from the origin in the PCA biplot across all vegetative generation among other entries in the experiment. However, their repeated position should not be described as proof of stability or superior performance. Rather, these entries can be regarded as promising candidates for further evaluation based on their multivariate phenotypic performance.

Overall, the result from genetic-variability parameters, correlation analysis, PCA, PCA biplot, and cluster analysis provided complementary information about the phenotypic structure of the evaluated putative mutant populations. The agreement among these analyses strengthens the basis for identifying entries with distinct and potentially desirable trait combination

5. CONCLUSION

Gamma irradiation induced substantial phenotypic variation among sweetpotato populations evaluated across three sequential generations. The identified LD_{50} values were 30.43 Gy for Alamura, 31.74 Gy for Kabode, and 33.58 Gy for Awassa-83, indicating varietal differences in radio sensitivity. Substantial variation was observed in qualitative, quantitative traits, and the estimates of GCV, PCV, broad-sense heritability, genetic advance, and GAM indicated considerable selection potential for several quantitative traits under the experimental conditions. Correlation, principal component, and hierarchical cluster analyses provided complementary information on relationships among traits and multivariate differentiation of the putative mutant lines.

The multivariate selection procedure resulted in the advancement of 254 putative mutant entries from M1V1 to M1V2 following exclusion of cluster 5, and 175 entries from M1V2 to M1V3 following exclusion of cluster 3. In M1V3, the 175 putative mutant entries were retained for further evaluation. Putative mutant lines such as AL3540, AL356, and AL355 were repeatedly identified as phenotypically divergent in the multivariate analyses and therefore warrant further evaluation.

Overall, the study demonstrates the usefulness of gamma irradiation combined with multivariate phenotypic characterization for generating and evaluating diverse sweetpotato populations. The identified putative mutant lines provide promising materials for further breeding research. However, confirmation of their genetic stability, agronomic superiority, and breeding value requires additional replicated evaluation across generations and environments.

Ethics approval and consent to participate Not applicable.

Consent for publication Not applicable.

Data availability and materials

The datasets used during the current study are available from the corresponding author upon reasonable request.

Conflicts of interest

The Authors declare no competing interest

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Authors' contributions

S.E.G. design the work, data collection, analyzing and interpretation of the data, manuscript writing, M.T.T. design the work, interpretation and manuscript editing, A.G.R. design the work, manuscript editing. All the authors read and decided for submission.

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Appendix Table

Appendix Table 1: Analysis of variance presented as mean squares (MS) and probability (Sig.) of the evaluated traits for 293 M1V1 mutants and their corresponding parents.

Taits	Source of Variation						
M1V1	Treatment	Block	Control	Augmented	Cont vs aug	Residual	CV%
DF	295	9	2	292	1	18	
PT	0.65***	0.06***	0.033	0.59***	19.11***	0.07	4.9
SHL	0.89***	0.89ns	11.2***	0.82***	0.4*	0.09	8.93
TL	2.87*	0.91ns	38.7***	2.57*	18.18**	1.22	17.7
SD	45.36***	5.02***	0.70	38.84***	2036.75***	0.48	4.25
FD	137.4***	22.67ns	620.4***	133.9***	178.77**	19.36	4.60
BN	4.82***	0.44ns	14.03***	4.75***	5.41**	0.48	14.0
LN	360.55***	2.45ns	873.4***	351.6***	1949.06***	3.91	4.7
VL	954.2***	4.8ns	960.7***	858.9***	28781.8***	5.0	4.5
Chla	3.28***	0.29ns	10.79***	3.24***	0.59ns	0.74	12.8
Chlb	3.97***	0.22ns	5.11**	3.98***	0.022ns	0.80	18.48
TC	10.57**	0.66 ns	24.02**	10.51**	0.39ns	2.8	14.56
CAR	2.1*	1.0ns	5.95	2.07	3.23	0.87	26.44

*Note: where ns= non significance, * , **, *** significance level at p<0.05,<0.01, < 0.001 respectively, SD=days to sprouting, FD=days to flowering, BN= branch number, LN=leaf number, VL= vine length, Chla=chlorophyll a, Chlb= chlorophyll b, TC=total chlorophyll, CAR=carotenoid,*

Appendix Table 2: Analysis of variance presented as mean squares (MS) and probability (Sig.) of the evaluated traits for 254 M1V2 mutants and their corresponding parents.

Taits M1V2		Source of Variation						
		Treatment	Block	Control	Augmented	Cont vs aug	Residual	CV%
	DF	256	8	2	253	1	16	
PT		0.69***	0.23ns	2.48***	0.63***	14.9***	0.11	5.54
SHL		0.89***	0.08ns	10.33***	0.82***	0.14***	0.08	5.96
TL		2.92*	0.82ns	32.26***	2.37*	3.92ns	3.46	17.45
FD		326.4***	58.9ns	2144.4***	300.3***	3276.7***	31.9	5.83
BN		3.43*	1.39ns	0.93ns	3.45*	4.87ns	1.34	23.16
LN		410.1***	182.0*	201.6*	396.9***	4146.4***	53.3	11,79
VL		1642***	131ns	16984***	1264***	66469***	153	16.10
Chla		3.04**	0.32ns	11.22***	2.9***	22.93	0.69	10.84
Chlb		4.23**	1.17ns	18.35***	3.94**	49.27***	1.22	16.33
TC		9.96***	1.75ns	57.99***	9.1***	139.0***	1.97	9.75
CAR		1.93***	0.40ns	10.77***	1.86***	0.54ns	0.35	22.47
Fv/Fm		0.04***	0.01ns	0.07***	0.03***	0.2***	0.007	12.64

*Note: where ns= non significance, *, **, *** significance level at p<0.05,<0.01, < 0.001 respectively, FD=days to flowering, BN= branch number, LN=leaf number, VL= vine length, Chla=chlorophyll a, Chlb= chlorophyll b, TC=total chlorophyll, CAR=carotenoid, Fv/Fm= chlorophyll florescence*

Appendix Table 3: Analysis of variance presented as mean squares (MS) and probability (Sig.) of the evaluated traits for 175 M1V3 mutants and their corresponding parents.

Taits M1V3		Source of Variation						
		Treatment	Block	Control	Augmented	Cont vs aug	Residual	CV%
	DF	177	5	2	174	1	10	

PT	0.44**	0.08ns	1.56***	0.41**	4.34***	0.09	4.9
SHL	0.87***	0.1ns	6.5***	0.8***	0.05ns	0.1	6.61
TL	2.9***	0.09ns	37.56***	2.55***	5.57***	0.09	4.99
FD	327.04***	7.12*	1996.06***	309.4***	58.84**	5.32	2.59
BN	3.58*	0.27ns	2.16ns	3.62*	0.39ns	1.02	19.58
LN	290.4***	3.7ns	560.9***	265.9***	4015.9***	1.2	1.75
VL	1387***	7ns	16603***	889***	57665***	21	6.17
Chla	4.00***	1.53ns	4.66**	3.89***	21.57***	0.54	9.56
Chlb	3.94***	1.44**	6.82***	3.88***	7.46***	0.17	6.41
TC	11.76***	5.19**	22.64***	11.41***	51.29***	0.63	5.58
CAR	2.22***	0.16ns	3.81***	2.07***	25.06***	0.23***	14.91
Fv/Fm	0.03***	0.001ns	0.04***	0.03***	0.26***	0.001	5.46
TN	4.11***	1.79***	9.39***	3.76***	54.48***	0.12	7.94

*Note: where ns= non significance, * , **, *** significance level at p<0.05,<0.01, < 0.001 respectively, FD=days to flowering, BN= branch number, LN=leaf number, VL= vine length, Chla=chlorophyll a, Chlb= chlorophyll b, TC=total chlorophyll, CAR=carotenoid, Fv/Fm= chlorophyll fluorescence, TN= tuber number*

Supplementary Tables and Figures

Table S1: Experimental design summary for each vegetative generation of the sweetpotato putative mutant population.

Generation	Total sample	Irradiated line	No of checks	No of blocks	No of plots per block	No of plots in last block
M1V1	323	293	30	10	33	27
M1V2	281	254	27	9	32	25
M1V3	193	175	18	6	33	28

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Table S2: List of morphological characteristics for sweetpotato putative mutant lines described by CIP-standard descriptor

No	Descriptor	Phenotype Vine characteristics
1	Plant type (PT)	3 Erect (< 20 cm); 5 Semi-erect (21 - 50 cm); 7 Spreading (51 - 100 cm); 9 Extremely spreading (>100 cm)

2	Leaf shape (SHL)	1 =Rounded; 2= Reniform (kidney-shaped); 3= Cordate (heart-shaped); 4 =Triangular; 5= Hastate; 6 Lobed; 7= Almost divided
3	Leaf lobe type (TL)	3= No lateral lobes (entire); 4= Very slight (teeth); 5= Slight; 6 =Moderate; 8= Deep; 9= Very deep

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GENERATION
M1V1
Cluster-1 (31)
AL151, AL152, AL153, AL154, AL251, AL252, AL2532, AL2536, AL2540, AL351, AW1516, AW151, AW1518, AW1535, AW1537, AW2513, AW3534, AW3544, K151, K152, K1523, K1524, K1525, K1526, K153, K1547, K251, K252, K253, K254, K356
Cluster-2 (59)
AL3511, AL3538, AL3546, AW251, AW2514, AW2517, AW2521, AW2522, AW2523, AW2524, AW2525, AW2527, AW2528, AW2529, AW253, AW2531, AW253, AW2534, AW2539, AW254, AW2540, AW2541, AW2542, AW2543, AW2544, AW2546, AW2549, AW255, AW2550, AW256, AW257, AW259,AW351, AW3513, AW3514, AW3515, AW3517, AW3519, AW3520, AW3521, AW3522, W3524, AW3528, AW3529, AW3537, AW3539, AW354, AW3542, AW3543, AW3545, AW3548, AW355, AW356, AW358, K3515, K3525, K3528, K3533, K3534
Cluster-3 (132+3)
AL, AL1510, AL1511, AL1512, AL1514, AL1515, AL1518, AL1519, AL1525, AL1527, AL1528, AL1529, AL1532, AL1533, AL1534, AL1537, AL1538, AL1540, AL1541, AL1542, AL1545, AL1546, AL1548, AL1549, AL155, AL1550, AL156, AL157, AL158, AL159, AL2510, AL2511, AL2512, AL2514, AL2515, AL2519, AL2524, AL2525, AL2526, AL2528, AL2531, AL254, AL255, AL257, AL258, AL259, AL3522, AL3523, AL3529, AL3547, AW, AW151, AW1510, AW1511, AW1514, AW1519, AW152, AW1520, AW1522, AW1525, AW1526, AW1528, AW1531, AW1533, AW1534, AW1538, AW1539, AW1541, AW1544, AW1545, AW1546, AW1547, AW1548, AW1549, AW155, AW1550, AW158, AW2519, AW252, AW2526, AW2538, AW2545, AW258, K, K1512, K1513, K1514, K1515, K1516, K1519, K1520, K1521, K1527, K1528, K1529, K1530, K1531, K1532, K1535, K1537, K1538, K1539, K1541, K1543, K1544, K1545, K155, K157, K158, K2512, K2513, K2514, K2520, K2521, K2523, K2524, K2525, K2526, K2528, K2529, K2532, K2533, K2535, K2536, K2537, K2540, K2542, K2544, K2546, K255, K2550, K256, K257, K258, K259
Cluster-4 (32)
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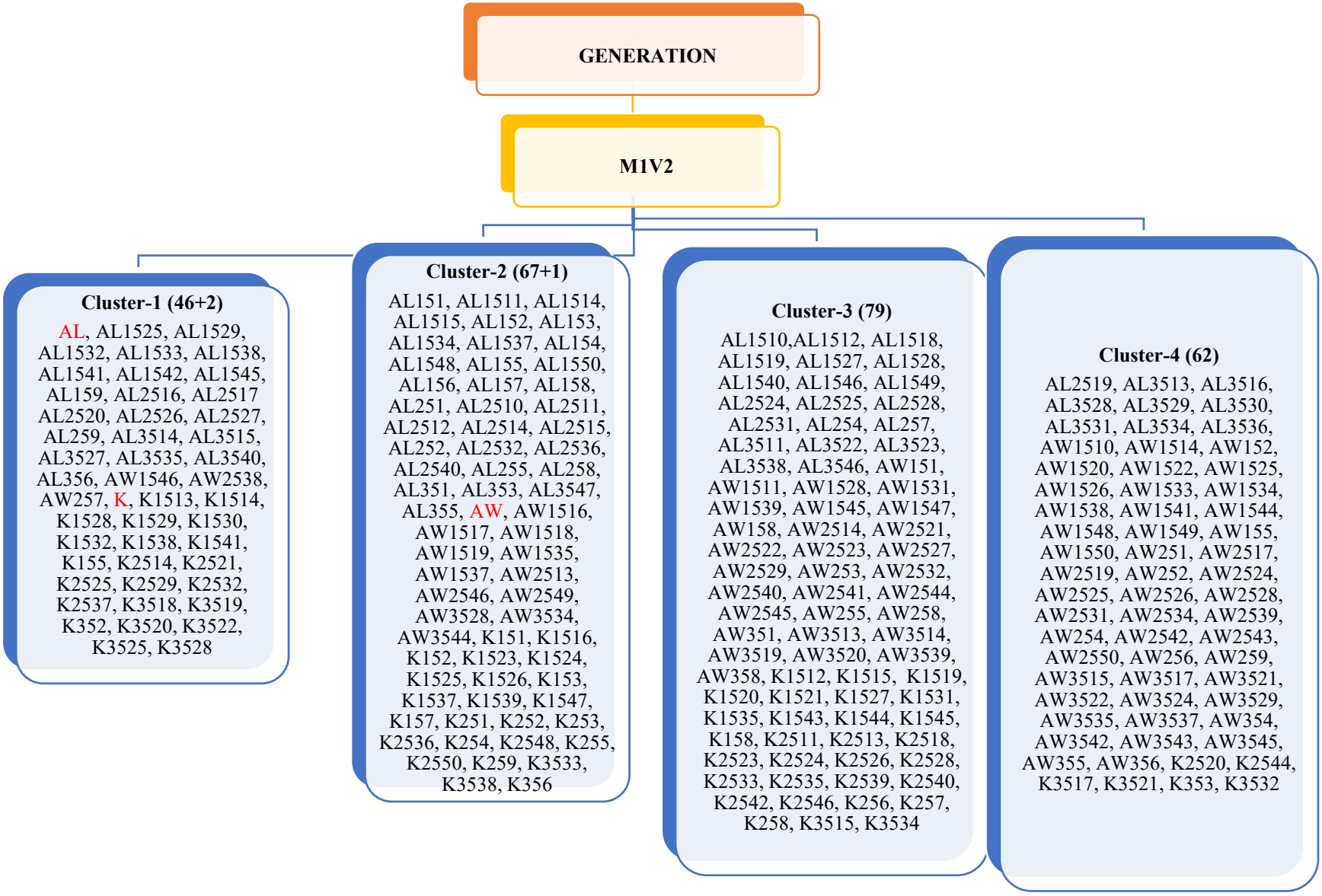
Cluster-5 (39)
AL1517, AL1520, AL1526, AL1531, AL1536, AL1539, AL1544, AL1547, AL2529, AL2530, AW1512, AW1523, AW1524, AW1527, AW1529, AW153, AW1536, AW1540, AW1542, AW159, K1511, K1517, K1518, K1522, K1533, K1534, K1536, K154, K1540, K1542, K1546, K156, K2511, K2522, K2534, K2538, K2543, K2547, K2549

951


GENERATION
M1V2
Cluster-1 (46+2)
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Cluster-2 (67+1)
AL151, AL1511, AL1514, AL1515, AL152, AL153, AL1534, AL1537, AL154, AL1548, AL155, AL1550, AL156, AL157, AL158, AL251, AL2510, AL2511, AL2512, AL2514, AL2515, AL252, AL2532, AL2536, AL2540, AL255, AL258, AL351, AL353, AL3547, AL355, AW, AW1516, AW1517, AW1518, AW1519, AW1535, AW1537, AW2513, AW2546, AW2549, AW3528, AW3534, AW3544, K151, K1516, K152, K1523, K1524, K1525, K1526, K153, K1537, K1539, K1547, K157, K251, K252, K253, K2536, K254, K2548, K255, K2550, K259, K3533, K3538, K356
Cluster-3 (79)
AL1510,AL1512, AL1518, AL1519, AL1527, AL1528, AL1540, AL1546, AL1549, AL2524, AL2525, AL2528, AL2531, AL254, AL257, AL3511, AL3522, AL3523, AL3538, AL3546, AW151, AW1511, AW1528, AW1531, AW1539, AW1545, AW1547, AW158, AW2514, AW2521, AW2522, AW2523, AW2527, AW2529, AW253, AW2532, AW2540, AW2541, AW2544, AW2545, AW255, AW258, AW351, AW3513, AW3514, AW3519, AW3520, AW3539, AW358, K1512, K1515, K1519, K1520, K1521, K1527, K1531, K1535, K1543, K1544, K1545, K158, K2511, K2513, K2518, K2523, K2524, K2526, K2528, K2533, K2535, K2539, K2540, K2542, K2546, K256, K257, K258, K3515, K3534
Cluster-4 (62)
AL2519, AL3513, AL3516, AL3528, AL3529, AL3530, AL3531, AL3534, AL3536, AW1510, AW1514, AW152, AW1520, AW1522, AW1525, AW1526, AW1533, AW1534, AW1538, AW1541, AW1544, AW1548, AW1549, AW155, AW1550, AW251, AW2517, AW2519, AW252, AW2524, AW2525, AW2526, AW2528, AW2531, AW2534, AW2539, AW254, AW2542, AW2543, AW2550, AW256, AW259, AW3515, AW3517, AW3521, AW3522, AW3524, AW3529, AW3535, AW3537, AW354, AW3542, AW3543, AW3545, AW355, AW356, K2520, K2544, K3517, K3521, K353, K3532

952

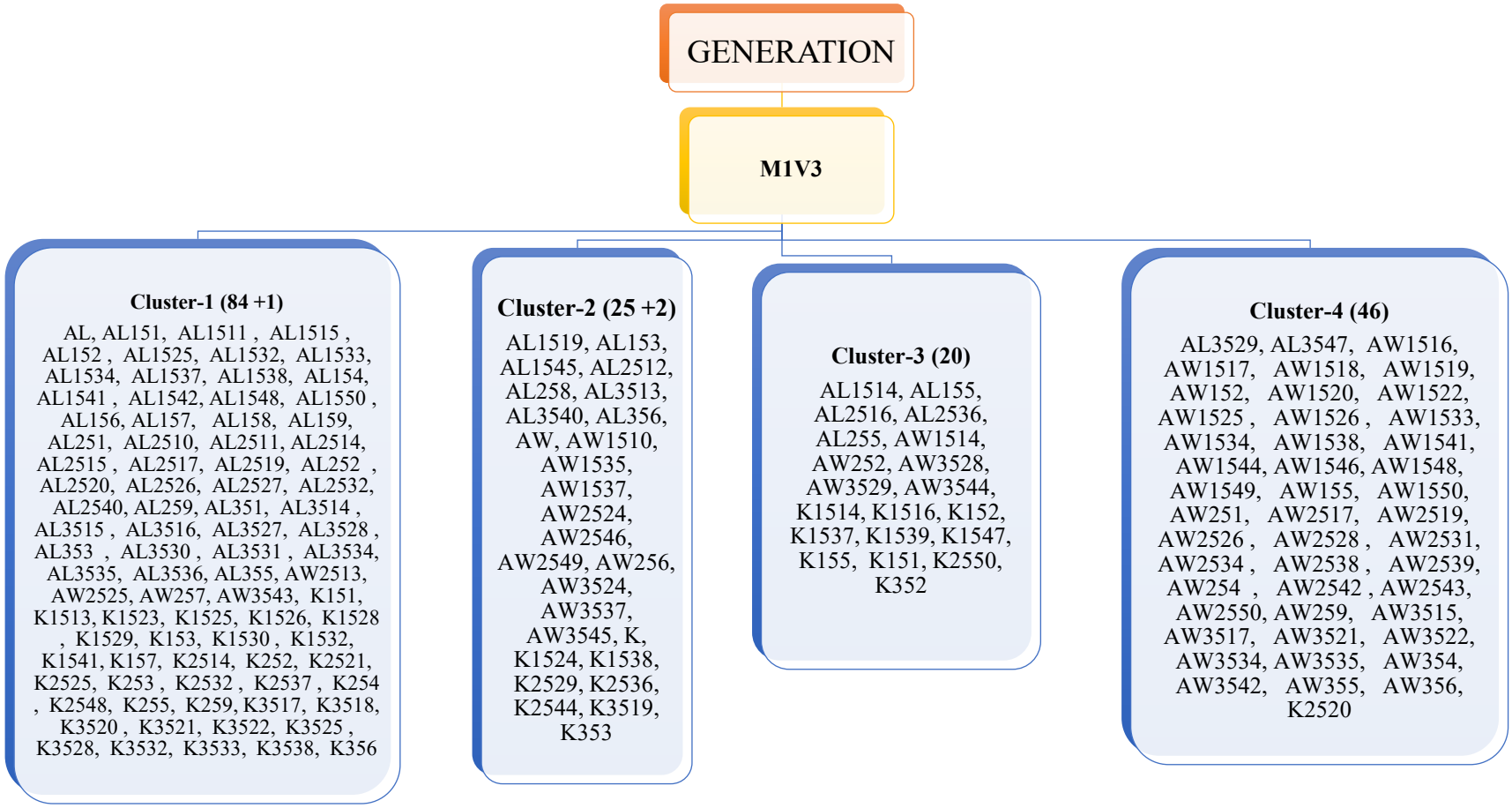

Figure S1: List of irradiated and control sweetpotato lines through generations and clusters

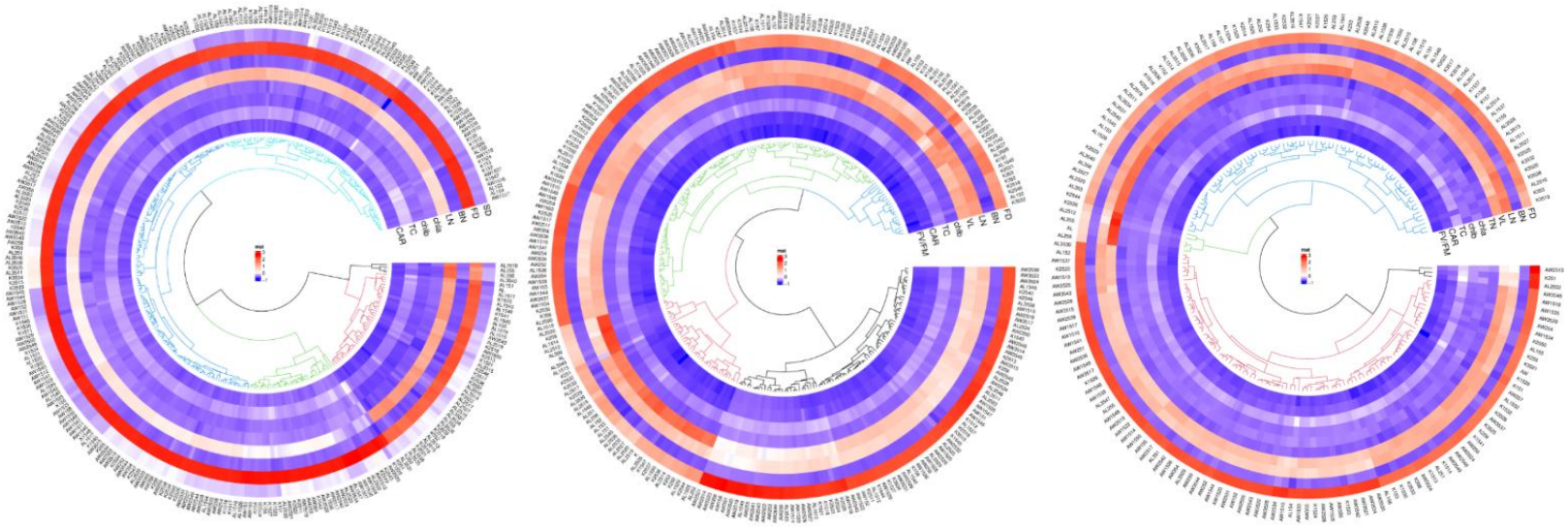

Figure S2. Heatmapdendrogram of 293, 254, and 175 sweetpotato putative mutant lines and their controls revealed by cluster analysis based on 8, 8, and 10 traits, respectively per generations

Supplementary Files

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- supportivematerials.docx