

**ORIGINAL RESEARCH**

## Assessment of Genetic Divergence in Mutant Lines of Tomato (*Solanum lycopersicum* L.)

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**Abstract:**

**Introduction:** Mutation breeding is the process of exposing seeds to chemicals or radiation in order to generate mutants with desirable traits.

**Aim:** This study is aimed at assessing the genetic variability among mutant lines of tomato (*Solanum lycopersicum* L.) generated from a variety of tomato (Roma VF) using two different chemo-mutagens.

**Materials and Method:** The collected seeds were exposed to different concentrations of Sodium azide and Colchicine with varied period of exposure. The seeds were planted and selected 49 positive mutant lines were assessed for morphological genetic variability and yield. 18 mutant lines that produced fruits were tagged and selected. The fruits of the selected mutants were harvested and the seeds (M2 seeds) were subsequently planted for divergence analysis. Ten quantitative characters and twenty qualitative characters were scored using IPGRI standard tomato descriptor. The potted experiment was laid out in the Green House, using Randomized Block Design (RBD) with three replications.

**Result:** The results of this study revealed a high genetic divergence among the mutant lines in both quantitative and qualitative characters. There was significant LSD (0.05) for Germination percentage (7.66), Plant height at maturity (7.05) and Number of leaves at maturity (4.56). The yield (fresh fruit weight) varied significantly, ranging from 10.00g for LeMT29 to 319.70g for LeMT7 respectively. Fruit and plant qualitative characters equally exhibit variation.

**Conclusion:** These observations suggest the existence of genetic variability among the different mutant tomato lines. Further selection and field trials is recommended to identify suitable and desirable lines for possible variety release.

**To Keywords:** Mutant, Tomato, Colchicine, Sodium azide, genetic variability.

All co-authors agreed to have their names listed as authors.

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## 1. INTRODUCTION

Mutation breeding is the process of exposing seeds to chemicals or radiation in order to generate mutants with desirable traits. From 1930–2007 more than 2,540 mutagenic plant varieties have been released that have been derived either as direct mutants (70%) or from their progeny (30%) [1]. Crop plants account for 75% of released mutagenic species with the remaining 25% being ornamentals or decorative plants [2]. In order to speak more clearly about mutations and their potential for crop improvement, it would seem desirable to have different terms at least for (a) the phenotypic alteration and (b) the various underlying molecular and numerical changes. But in any case, a mutation has to be phenotypically expressed to be selectable; all other mutations are only of scientific interest [3]. Mutations are the tools used by the geneticist to study the nature and function of genes which are the building blocks and basis of plant growth and development, thereby producing raw materials for genetic improvement of economic crops [4]. It is a powerful and effective tool in the hands of plant breeders especially for autogamous crops having narrow genetic base [4, 5]. During the past 70 years, more than 3,222 mutant cultivars from 175 plant species including ornamentals, cereals, oilseeds, pulses, vegetables, fruits and fibers have been officially released in 50 countries all over the World [1, 6, 7, 8].

The cultivated tomato (*Solanum lycopersicum* L.) is the most popular garden vegetable and is the second most important vegetable crop in the world in terms of consumption per capita [9, 10]. In 2004, tomato assumed the position of one of the most important fruits in terms of Worlds' vegetable produced [11]. Tomato is grown in almost every corner of the planet. On global basis, it is planted 4.6 million hectares of agricultural lands with a total production of 125.5 million metric tons [12]. Nigeria is the 14th largest producer of tomatoes in the world and second only to Egypt in Africa at 1.51 million metric tons valued at N87 billion (\$556.1 million) with a cultivated area of 264,430 ha [13]. Both the wet and dry season cropping system contributes immensely to the national requirement. But the bulk production is from the dry season cropping system grown yearly under irrigation system [14]. Tomato belongs to the *Solanaceae* family, which includes 3,000 species with origins in both the Old (eggplant in China and India) and New World [15].

Several breeders have studied genetic diversity in tomato germplasm for improvement of various growth and yield related traits [16, 17, 18] but Nigeria has a narrow tomato genetic base, therefore the main aim of this study was to induce variability in a known genotype of tomato (ROMA VF) using two chemical mutagens (Sodium azide and Colchicine) with the specific objectives of selecting positive mutants, evaluation of the selected mutant lines to determine the extent of genetic variability and identification of promising mutant lines for future trials.

## 2. MATERIAL AND METHODS

Dried seeds of tomato (var. ROMA VF) collected from the Institute of Agriculture Research and Training (IART) Moore Plantation Ibadan, Oyo state, Nigeria was the foundation seed used in this study. Tomato seeds collected were pre-soaked in water for 24 hours. The presoaked seeds were treated with different concentration of Sodium azide ( $1.0 \times 10^{-3}$  mol,  $2.5 \times 10^{-3}$  mol and  $5.0 \times 10^{-3}$  mol) and Colchicine (0.05% and 0.1%) with varied period of exposure (15 min, 30 min and 45 min). The treated seeds were planted in rows having 10-plants per row keeping row-to-row and plant-to-plant distances of 60 cm and 30 cm, respectively with the untreated seeds serving as control.

49 positive mutant lines were identified and tagged as appropriate. The selected 49 positive mutant lines were assessed for morphological genetic variability and yield. The potted experiment was laid out in the greenhouse of the department of Botany, Lagos State University, Ojo Lagos using Randomized Block Design (RBD) with three replications. The blocks contained 10 stands (pots) of each 49 mutant lines, making 30 stands per line (these were the M1 plants). The between and within row spacing of 60cm and 30cm respectively was maintained. 18 mutant lines that produced fruits were tagged and selected. This is represented in Table 1. The fruits of the selected mutants were harvested and the seeds (M2 seeds) were subsequently planted for divergence analysis.

Ten quantitative characters and twenty qualitative characters were scored for using the standard descriptor for tomato [19]. Quantitative data scored for includes; plant height at maturity, number of leaves per plant etc., while qualitative characters include growth habit, stem pubescence etc. Quantitative data collected was subjected to statistical analysis using the Fisher's Least Significance Difference (LSD) test [20].

## 3. RESULTS AND DISCUSSION

Considerable variation was observed for all studied traits among the mutant lines. Their phenotypic variations were estimated and shown in different tables and figures. The mean, standard deviation and least significant difference (LSD) of the 10 quantitative characters analyzed for the 18 positive mutant lines that produced fruits are presented in Tables 2. A look at Table 2 reveals that the mean yield (fresh fruit) for the 18 mutant lines (M2) ranged from 10.00g per plant for LeMT29 to 319.70g per plant for LeMT7, the mean plant height at maturity ranged from 46.54cm for LeMT11 to 117.72cm for LeMT49. The average number of nodes at maturity also ranged from 1.20 for LeMT27 and LeMT47 to 5.25 in LeMT49.

The qualitative characters of Roma VF (control) and the 18 positive mutant lines are presented in Table 3. There was high level of diversity in some characters such as fruit shape, growth habit, stem pubescence, folia density, leaf altitude and degree of leaf dissection. The

fruit shape of Roma VF (control) is highly rounded. However, the mutagens had effect on the gene(s) controlling fruit shape leading to high level of diversity observed in fruit shape from high rounded in Roma VF to pointed, cylindrical, heart shape, pyriform, slightly flattened and rounded observed in the mutants. The leaf colour, colour of immature fruit and fruit shoulder shape exhibit moderate level of diversity among the mutant lines against the control. While shape of pistil scar, fruit radial cracking and fruit cross-sectional shape exhibited low level of diversity among the mutants against the control.

Figure 1 is a graphical representation of plant height at maturity among the 18 selected mutant lines against the control. Figure 2 is graphical expression of yield per plant among the 18 positive mutant lines against the control. Closer look at figure 1 and 2 revealed variation among the selected mutant lines as well as from the control (Roma VF) used in the study. LeMT7 had the highest yield (319.7g), followed by LeMT49 (240g), LeMT39 (187.75g), LeMT11 (155.8g) and LeMT2 (130.12g) as against the Roma VF (control) with yield of 125.15g. LeMT28 and LeMT29 had the lowest yield of 10.80g and 10.00g respectively.

## DISCUSSION

The results of this study indicate a high genetic divergence among the mutant lines against the control in both quantitative and qualitative characters. There was significant difference for germination percentage, plant height and number of leaves among the mutant lines. The yield varied significantly among the positive mutant lines. The highly significant differences observed using Fisher's least significant difference (LSD) [20] for Germination percentage, Plant height at flowering and number of leaves at flowering among the genotypes evaluated was indication of great deal of variability with respect to these characters. In the same vein, the observed significant variation in the yield among the 18 positive mutant lines also supports the earlier claim that there is a great deal of variability among the genotypes. The observed divergence among the positive mutant lines in reference to both quantitative and qualitative characters was in line with the work of [4] on tomato using Sodium azide and it further confirms that generally, Sodium azide and colchicine are very effective in inducing mutations in tomato. A thorough analysis on quantification of phenotypic variation due to mutagenic effect has also been reported in chickpea [21].

Fruit weight is a quantitatively inherited character that is controlled by many genetic loci; some may have a large effect while others have small effect [22, 23, 24]. High fruit weights observed may be as a result of the effect of the mutagens on the allele fw 2.2 which influences fruit weight [25]. This allele acts as a regulator of cell division in larger size fruits of tomato [26]. The differences in fruit size observed may be due to the regulators of cell division and cell size acting after anthesis [27].

No significance effect was observed in this population of tomato for number of branches per plant, number of nodes, leaf blade length, and petiole length and as such these characters cannot be used for selection in subsequent improvement program. This observation was consistent with earlier reports by Adamu et al. [28, 29] on mutagenic study on groundnut and tomato using gamma rays and that of Sheeba et al. [30] using gamma rays and EMS on *Sesamum indicum* further confirms that the effects of mutagens on these traits were dose dependent.

There was wide array of variations observed in respect of fruit and plant qualitative characters. The variations were easily recognizable with visual observation. These variations may be as a result of the effect of the mutagens used in this study on the alleles controlling these characters.

## 4. CONCLUSION

In conclusion, this study has shown that variation can be easily induced in crop plant using chemical mutagens such as Colchicine and Sodium azide. The level of variation observed after treatment buttress the earlier claim that mutation can raw materials for genetic improvement of economic crops. A number of the positive mutants selected perform better than the original variety (Roma VF).

The following selected positive mutants LeMT7, LeMT49, LeMT39 and LeMT11 has been identified as good lines that can form basis for subsequent improvement program in tomato. Subsequent evaluation can further screen these lines with view to releasing them as a new variety.

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## COMPETING INTERESTS

We declare that there are no competing interests with anyone on this research work and the manuscript.

## AUTHORS' CONTRIBUTIONS

Sunday C. Makinde, Regland M. Onyemeka and Opeyemi Oyetunji designed the study. Regland M. Onyemeka performed the statistical analysis, managed the literature searches and wrote the first draft of the manuscript. Sunday C. Makinde, Opeyemi Oyetunji, Abisola Ogunba and Muinat Asuni reviewed the first draft before submission.

**Table 1: Selected 18 mutant lines (M2) and their coding**

Serial Number	Lines	TREATMENTS
1	LeMT1	NaN <sub>3</sub> (1.0 × 10 <sup>-3</sup> )/15min
2	LeMT2	NaN <sub>3</sub> (1.0 × 10 <sup>-3</sup> )/15min
3	LeMT6	NaN <sub>3</sub> (1.0 × 10 <sup>-3</sup> )/45min
4	LeMT7	NaN <sub>3</sub> (2.5 × 10 <sup>-3</sup> )/15min
5	LeMT10	NaN <sub>3</sub> (2.5 × 10 <sup>-3</sup> )/15min
6	LeMT11	NaN <sub>3</sub> (2.5 × 10 <sup>-3</sup> ) 30min
7	LeMT23	NaN <sub>3</sub> (5.0 × 10 <sup>-3</sup> )/30min
8	LeMT24	NaN <sub>3</sub> (5.0 × 10 <sup>-3</sup> )/30min
9	LeMT25	NaN <sub>3</sub> (5.0 × 10 <sup>-3</sup> )/45min
10	LeMT26	NaN <sub>3</sub> (5.0 × 10 <sup>-3</sup> )/45min
11	LeMT27	NaN <sub>3</sub> (5.0 × 10 <sup>-3</sup> )/45min
12	LeMT28	NaN <sub>3</sub> (5.0 × 10 <sup>-3</sup> ) / 45min
13	LeMT29	Colchicine (0.1%)/15min
14	LeMT30	Colchicine (0.1%)/15min
15	LeMT33	Colchicine (0.1%)/30min
16	LeMT39	Colchicine (0.1%)/45min
17	LeMT47	Colchicine (0.05%)/45min
18	LeMT49	Colchicine (0.05%)/45min

**Table 2: Population means, SD and LSD for quantitative characters of the control and 18 positive mutant lines of Tomato (*Solanum lycopersicum*).**

Lines	PH (cm)		ID (cm)		PL (cm)		SG (cm)		NL (cm)		LL (cm)	
	GP	at	NB at	NN at	at	at	BL (cm) at	at	at	at	CN at	Yield
	(%)	maturity	maturity	maturity	maturity	maturity	maturity	maturity	maturity	maturity	maturity	(g)
Control												
(Roma VF)	100.00	52.40	5.00	20.20	4.98	6.34	27.60	2.56	16.20	19.08	5.00	125.15
LeMT1	90.00	71.32	2.40	15.00	4.38	4.98	19.82	2.98	25.20	22.26	5.00	106.06
LeMT2	90.00	54.56	1.40	14.60	3.68	4.80	14.80	2.74	18.80	21.64	5.00	130.12
LeMT6	80.00	69.68	3.20	20.60	4.72	7.02	17.40	3.24	34.20	24.38	5.00	22.96
LeMT7	70.00	78.78	3.00	16.80	5.08	5.48	20.46	3.32	32.60	24.30	5.00	319.70
LeMT10	90.00	50.52	5.00	13.40	3.46	4.87	14.48	2.76	17.00	18.80	5.00	92.97
LeMT11	65.00	46.54	2.00	14.00	4.26	3.90	10.66	2.44	18.00	15.34	5.00	155.80
LeMT23	55.00	80.68	3.50	18.50	3.35	5.00	17.93	3.03	24.75	23.24	5.00	37.94
LeMT24	65.00	111.74	4.40	25.00	3.30	6.74	13.54	3.26	50.40	24.48	5.00	23.00
LeMT25	25.00	88.80	4.00	18.50	4.28	6.23	16.33	3.28	39.25	23.78	6.00	13.72
LeMT26	50.00	83.34	1.80	17.80	4.68	6.02	20.42	3.10	31.20	19.07	5.00	50.10
LeMT27	55.00	73.32	1.20	15.40	4.50	5.26	13.90	3.26	16.60	21.02	5.00	110.00
LeMT28	55.00	72.66	2.00	14.60	6.78	5.82	19.66	3.18	20.00	24.39	5.00	10.50
LeMT29	60.00	67.15	1.25	13.75	3.65	5.85	13.73	2.90	19.75	17.17	5.00	10.00
LeMT30	55.00	62.82	2.40	14.80	4.62	5.74	14.86	3.30	21.80	19.01	5.00	12.49
LeMT33	90.00	92.04	5.00	22.60	4.40	4.84	11.32	3.14	42.20	19.18	6.00	57.00
LeMT39	80.00	57.36	2.00	15.60	6.40	3.64	14.28	2.94	20.40	18.35	5.00	187.75
LeMT47	75.00	71.82	1.20	19.00	4.44	4.74	15.06	2.86	20.40	19.00	5.00	83.72
LeMT49	80.00	117.72	5.25	26.25	5.60	4.73	15.58	12.70	51.50	21.23	5.00	240.00
Mean	68.33	75.05	2.83	17.57	4.53	5.31	15.79	3.58	28.00	20.92	5.11	92.44
SD	17.74	19.07	1.39	3.87	0.96	0.89	2.95	2.29	11.36	2.81	0.32	87.54
LSD(0.05)	12.48*	13.41*	0.98	2.72	0.68	0.62	2.07	1.61	7.99	12.48*	1.98	61.7*

\* = Significant LSD (0.05)

Quantitative characters scored for in this study, how they are scored and unit of measurement

NB = Number of Branches/ plant at maturity (determined by counting)	NB = Number of Branches/ plant at maturity (determined by counting)
GP = Germination Percentage (%)	NN = Number of Nodes/ plant at maturity (determined by counting)
ID = Inter-nodal Distance (measuring fourth to seventh internodes) (cm)	PH = Plant Height at maturity (measured from the base to stem apex) (cm)
LL = Total Leave Length (measured from the base of petiole to leaf apex) (cm)	PL = Leaf Petiole Length (Sample leaves on fourth to seventh nodes) (cm)
LN = Number of Leaves/ plant (determined by counting)	SG = Stem Girth at maturity (measured on the ground level) (cm)

**Table 3: Qualitative characteristics of the 18 positive mutant lines of Tomato (*Solanum lycopersicum*)**

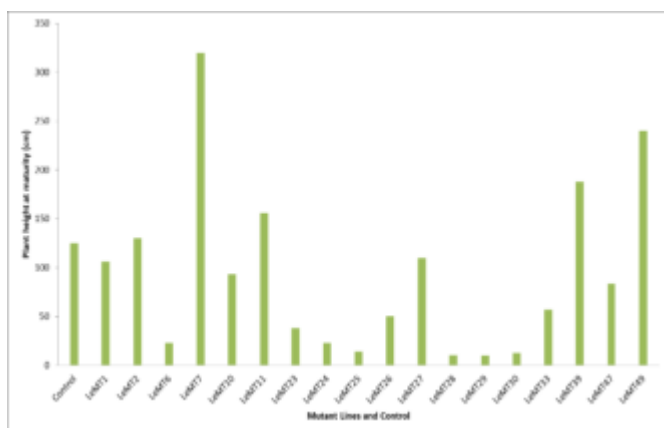
LINE	TREATMEN T	G H	S P	FD	LA	L T	DL D	L C	CI	G S	F S	C R	C S	F B	S S	F F	FR C	SS F	S P S
Roma VF	Control	3	5	5	7	2	5	2	3	0	4	5	1	2	3	1	0	5	2
LeM T1	NaN <sub>3</sub> (1.0 × 10 <sup>-3</sup> )/15min	3	5	5	3	3	5	4	3	0	6	5	1	3	3	1	0	1	2
LeM T2	NaN <sub>3</sub> (1.0 × 10 <sup>-3</sup> )/15min	3	7	7	3	3	7	4	3	1	4	5	1	1	3	1	0	3	2
LeM T6	NaN <sub>3</sub> (1.0 × 10 <sup>-3</sup> )/45min	3	7	7	5	3	7	2	3	0	3	5	1	2	3	1	0	3	2
LeM T7	NaN <sub>3</sub> (2.5 × 10 <sup>-3</sup> )/15min	3	7	3	3	3	3	2	3	1	4	4	1	1	3	2	0	3	2
LeM T10	NaN <sub>3</sub> (2.5 × 10 <sup>-3</sup> )/15min	3	7	7	7	3	7	2	5	1	6	4	1	1	3	1	1	3	4
LeM T11	NaN <sub>3</sub> (2.5 × 10 <sup>-3</sup> )/30min	1	7	5	7	5	3	2	3	0	6	5	2	3	3	2	0	1	2
LeM T23	NaN <sub>3</sub> (5.0 × 10 <sup>-3</sup> )/30min	3	7	5	7	3	5	2	5	0	3	5	1	2	3	1	0	3	2

LeM T24	NaN <sub>3</sub> (5.0 × 10 <sup>-3</sup> )30min	3	3	3	5	3	3	3	3	0	4	5	1	1	3	2	0	5	2
LeM T25	NaN <sub>3</sub> (5.0 × 10 <sup>-3</sup> )45min	2	3	3	3	3	3	3	5	0	3	5	1	2	3	1	1	3	2
LeM T26	NaN <sub>3</sub> (5.0 × 10 <sup>-3</sup> )45min	3	5	5	3	3	5	2	3	0	6	4	1	2	3	1	1	3	2
LeMT2 7	NaN <sub>3</sub> (5.0 × 10 <sup>-3</sup> )/45min	1	5	5	3	3	3	2	5	1	6	4	1	2	3	3	0	7	2
LeMT2 8	NaN <sub>3</sub> (5.0 × 10 <sup>-3</sup> )/45min	3	7	3	5	3	5	2	3	0	6	4	1	3	3	2	0	3	2
LeMT2 9	Colchicine( 0.1%) 15min	1	5	3	7	3	5	2	5	1	6	4	1	3	3	1	0	1	2
LeMT3 0	Colchicine (0.1%) 15min	3	7	3	7	3	7	1	3	0	5	5	1	3	3	5	0	7	2
LeMT3 3	Colchicine (0.1%) 30min	3	3	7	7	3	7	2	7	1	3	5	1	2	3	1	1	1	2
LeMT3 9	Colchicine (0.1%) 45min	3	7	7	3	6	5	2	5	1	2	4	2	2	3	1	0	3	2
LeMT4 7	Colchicine (0.05%) 45min	2	3	3	3	1	3	2	3	0	6	4	2	3	3	1	0	1	2
LeMT4 9	Colchicine (0.05%) 45min	1	3	3	3	3	3	2	3	0	7	4	2	1	3	1	1	3	2

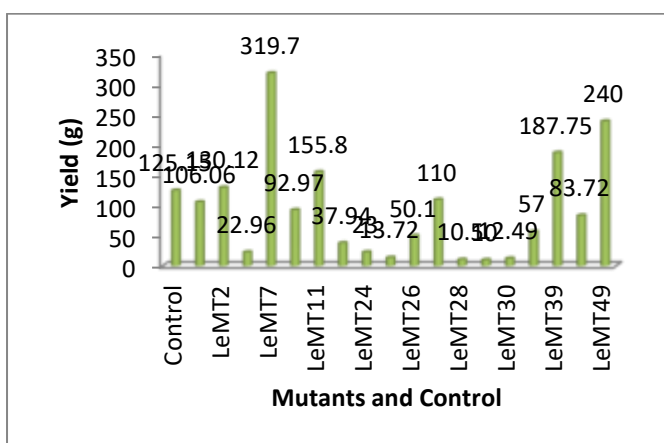
The qualitative characters were scored by physical observation and comparing with tomato standard descriptors by ipgri, 1996



CI = Colour of Immature Fruit  
 GH = Growth Habit  
 CR = Colour of Ripe Fruit  
 GS = Green Shoulder  
 CS = Fruit Cross-Sectional Shape  
 LA = Leaf Altitude  
 DLD = Degree of Leaf Dissection  
 LC = Leaf Colour  
 FB = Fruit Blossom End Shape  
 LT = Leaf Type  
 FC = Fruit Cracking  
 SSF = Shoulder Shape of Fruit  
 FD = Foliage Density  
 SP = Stem Pubescence  
 FF = Fruit Feature  
 SPS = Shape of Pistil Scar  
 FRC = Fruit Radial Cracking  
 SS = Seed Shape  
 FS = Fruit Shape



**Figure 1: bar chart comparing plant height of control and the 18 positive mutant lines of tomato (*solanum lycopersicum*).**



**Figure 2: bar chart comparing yield of the control and 18 positive mutant lines of tomato (*solanum lycopersicum*).**



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