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ASSESSMENT OF GENETIC DIVERGENCE IN MUTANT LINES OF TOMATO (*SOLANUM LYCOPERSICUM* L.)

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

Keywords— **Mutant, Tomato, Colchicine, Sodium azide, genetic variability**

I. 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 varietals have been released that have been derived either as direct mutants (70%) or from their progeny (30%) (Maluszynsk *et al*. 2000). Crop plants account for 75% of released mutagenic species with the remaining 25% being ornamentals or decorative plants (Ahloowali, 2004). 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 (Bahar and Samiullah, 1999). 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 (Adamu and Aliyu, 2007). It is a powerful and effective tool in the hands of plant breeders especially for autogamous crops having narrow genetic base

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(Adamu and Aliyu, 2007; Micke *et al*. 1985). 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 (Maluszynsk *et al*. 2000; Mashenkov, 1985; Chopra, 2005; Scossiroli, 1977).

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 capital (Nitish-Kumar *et al*., 2017 and FAO, 2005). In 2004, tomato assumed the position of one of the most important fruits in terms of Worlds' vegetable produced (FAO, 2005). 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 (FAOSTAT, 2008). 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 (CBN, 2013). 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 (Ojo *et al.* 2009). 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 (Knapp, 2002).

Several breeders have studied genetic diversity in tomato germplasm for improvement of various growth and yield related traits (El-Awady *et al*. 2012; qbal *et al.* 2014; Saleem *et al*. 2015) 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.

II. MATERIALS AND METHOD

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 (1.0×10^{-3} mol, 2.5×10^{-3} mol and 5.0×10^{-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. 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 (IPGRI, 1996). 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 (Gomez and Gomez, 1984).

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

Serial Number	Lines	Treatments
1	LeMT1	$NaN_3(1.0 \times 10^{-3})$/15min
2	LeMT2	$NaN_3(1.0 \times 10^{-3})$/15min
3	LeMT6	$NaN_3(1.0 \times 10^{-3})$/45min
4	LeMT7	$NaN_3(2.5 \times 10^{-3})$/15min
5	LeMT10	$NaN_3(2.5 \times 10^{-3})$/15min
6	LeMT11	$NaN_3(2.5 \times 10^{-3})$ 30min
7	LeMT23	$NaN_3(5.0 \times 10^{-3})$/30min
8	LeMT24	$NaN_3(5.0 \times 10^{-3})$/30min
9	LeMT25	$NaN_3(5.0 \times 10^{-3})$/45min
10	LeMT26	$NaN_3(5.0 \times 10^{-3})$/45min
11	LeMT27	$NaN_3(5.0 \times 10^{-3})$/45min
12	LeMT28	$NaN_3(5.0 \times 10^{-3})$/ 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

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III. RESULT

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 5.25in 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.

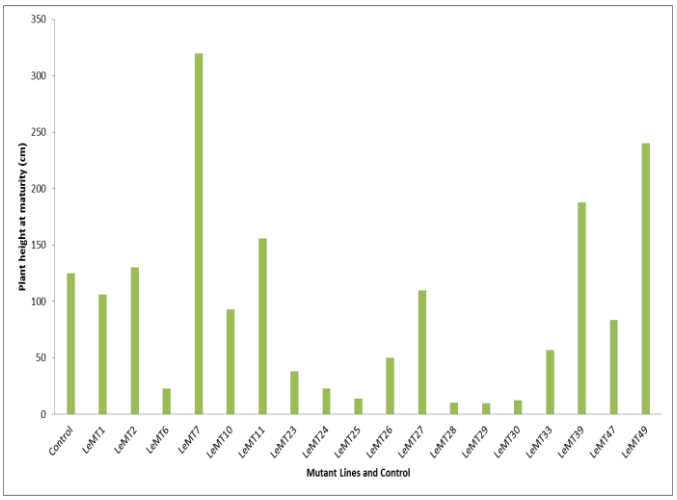

Figure 1: Bar chart comparing Plant height of control and the 18 positive mutant lines of Tomato (*Solanum lycopersicum*).

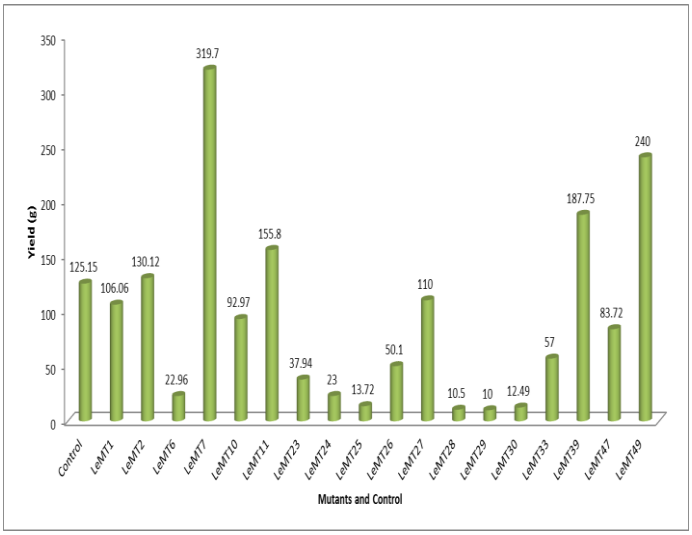

Figure 2: Bar chart comparing yield of the control and 18 positive mutant lines of Tomato (*Solanum lycopersicum*).

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

Lines	GP (%)	PH (cm) at maturity	NB at maturity	NN at maturity	ID (cm) at maturity	PL (cm) at maturity	BL (cm) at maturity	SG (cm) at maturity	NL (cm) at maturity	LL (cm) at maturity	CN at maturity	Yield (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

BL = Leaf Blade Length (measured along the midrib to the leaf apex) (cm)
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)

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

LINES	TREATMENT	GH	SP	FD	LA	LT	DLD	LC	CI	GS	FS	CR	CS	FB	SS	FF	FRC	SSF	SPS
Roma VF	Control	3	5	5	7	2	5	2	3	0	4	5	1	2	3	1	0	5	2
LeMT1	$NaN_3(1.0 \times 10^{-3})$/15min	3	5	5	3	3	5	4	3	0	6	5	1	3	3	1	0	1	2
LeMT2	$NaN_3(1.0 \times 10^{-3})$/15min	3	7	7	3	3	7	4	3	1	4	5	1	1	3	1	0	3	2
LeMT6	$NaN_3(1.0 \times 10^{-3})$/45min	3	7	7	5	3	7	2	3	0	3	5	1	2	3	1	0	3	2
LeMT7	$NaN_3(2.5 \times 10^{-3})$15min	3	7	3	3	3	3	2	3	1	4	4	1	1	3	2	0	3	2
LeMT10	$NaN_3(2.5 \times 10^{-3})$15min	3	7	7	7	3	7	2	5	1	6	4	1	1	3	1	1	3	4
LeMT11	$NaN_3(2.5 \times 10^{-3})$30min	1	7	5	7	5	3	2	3	0	6	5	2	3	3	2	0	1	2
LeMT23	$NaN_3(5.0 \times 10^{-3})$30min	3	7	5	7	3	5	2	5	0	3	5	1	2	3	1	0	3	2
LeMT24	$NaN_3(5.0 \times 10^{-3})$30min	3	3	3	5	3	3	3	3	0	4	5	1	1	3	2	0	5	2
LeMT25	$NaN_3(5.0 \times 10^{-3})$45min	2	3	3	3	3	3	3	5	0	3	5	1	2	3	1	1	3	2
LeMT26	$NaN_3(5.0 \times 10^{-3})$45min	3	5	5	3	3	5	2	3	0	6	4	1	2	3	1	1	3	2
LeMT27	$NaN_3(5.0 \times 10^{-3})$/45min	1	5	5	3	3	3	2	5	1	6	4	1	2	3	3	0	7	2
LeMT28	$NaN_3(5.0 \times 10^{-3})$/45min	3	7	3	5	3	5	2	3	0	6	4	1	3	3	2	0	3	2
LeMT29	Colchicine(0.1%) 15min	1	5	3	7	3	5	2	5	1	6	4	1	3	3	1	0	1	2
LeMT30	Colchicine (0.1%) 15min	3	7	3	7	3	7	1	3	0	5	5	1	3	3	5	0	7	2
LeMT33	Colchicine (0.1%) 30min	3	3	7	7	3	7	2	7	1	3	5	1	2	3	1	1	1	2
LeMT39	Colchicine (0.1%) 45min	3	7	7	3	6	5	2	5	1	2	4	2	2	3	1	0	3	2
LeMT47	Colchicine (0.05%) 45min	2	3	3	3	1	3	2	3	0	6	4	2	3	3	1	0	1	2
LeMT49	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
FB = Fruit Blossom End Shape
FC = Fruit Cracking
FD = Foliage Density
FF = Fruit Feature
FRC = Fruit Radial Cracking
FS = Fruit Shape
LC = Leaf Colour
LT = Leaf Type
SSF = Shoulder Shape of Fruit
SP = Stem Pubescence
SPS = Shape of Pistil Scar
SS = Seed Shape

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IV. DISCUSSION

The results of this study revealed 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) (Gomez and Gomez, 1984) 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 vain, the observed significant variation in the yield among the 18 positive mutant lines also supports the earlier claim. The observed divergence among the positive mutant lines in reference to both quantitative and qualitative characters was in line with the work of (Adamu and Aliyu, 2007) 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 was also reported in chickpea Laskar *et al.* (2015).

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 (Ben-Chaim *et al.* 2006; Doganlar *et al.* 2002; Grandillo *et al*. 1999). 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 (Frary *et al*. 2000). This allele acts as a regulator of cell division in larger size fruits of tomato (Nunoo *et al*. 2014). The differences in fruit size observed may be due to the regulators of cell division and cell size acting after anthesis (Paran, and Van der Knaap, 2007).

Not 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 report by Adamu *et al*. (2002, 2004) on mutagenic study on groundnut and tomato using gamma rays and that of Sheeba *et al*. (2005) using gammar rays and EMS on *Sesanum indicum* further confirms that the effects of mutagens on these traits were dose dependent.

There was wide array of variation 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. Similar conclusion had been drawn by Hanson, (2005) and Agong *et al.* (1997) in a study made on tomato.

V. CONCLUSION AND RECOMMENDATION

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

Recommendation

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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