Agro-Science Journal of Tropical Agriculture, Food, Environment and Extension
Volume 22 Number 2 (April 2023) pp. 73 - 77
ISSN 1119-7455

FIELD EVALUATION OF M4 PLANTS OBTAINED FROM INDUCED MUTATION OF TOMATO 'COBRA' VARIETY WITH ETHYL METHANE SULFONATE (EMS)

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ABSTRACT

Crop improvement through induced mutation has resulted in the development of new mutant varieties worldwide. Alkylating agents such as ethyl methane sulfonate (EMS) are widely used to induce mutations in plants due to their ability to cause changes in the nucleotide sequence. In this study, a field experiment was conducted at the University of Nigeria Nsukka in southeastern Nigeria to evaluate M4 plants of tomato variety 'Cobra' obtained from selections in the third generation (M3) plants in the field. Seeds of the cobra tomatoes were subjected to induced mutation using different concentrations of ethyl methane sulfonate (EMS). Before this study, selections based on desired horticultural traits were carried out in the first-generation mutant (M1), second generation mutant (M2), and third generation (M3). Eight different mutants were selected and evaluated in the field as M4 plants. Variations were observed in both the qualitative and quantitative traits studied. Average fruit weight of 1.118 kg per plant and 23.60 fruits per plant were recorded for variant 3 as against the parent plant that had 479 g per plant and produced 14 fruits per plant. Other promising mutants in terms of fruit yield per plant were variant 5 (1.079 kg) a variant 2 (1.069 kg). Remarkably, there was no incidence of blossom end rot in all the M4 variants evaluated.

Key words: crop breeding, crop improvement, plant generations, *Solanum lycopersicum*, variants

INTRODUCTION

Growing tomatoes in a developing country such as Nigeria is faced with a lot of challenges, ranging from lack of improved seeds to challenges of weather posed by global climate change to crop yield. Because of the ever-increasing demand for food and other agricultural products, crop improvement techniques must be more precise in developing improved crop varieties that would be pest and disease resistant. Therefore, keeping in view the nutritional and economic values of tomatoes (*Solanum lycopersicum* L.) and the continued demand for tomatoes and its product, crop improvement is needed the most (Laskar *et al.,* 2018). Mutation breeding is now widely used due to its potency in creating lots of variability in plants (Mir *et al.,* 2020) and has brought a lot of benefits in modern agricultural production as a method for crop improvement and the addition of new valuable traits to the existing varieties (Ahloowalia and Maluszynki, 2001). The use of induced mutations for crop improvement has yielded several mutants which have been used directly as new cultivars (Gottschalk and Wolf, 1983). Induced mutation in tomatoes is important for tomato breeding and crop improvement. Alkylating agents like ethyl methane sulfonate (EMS) are widely used to induce mutations in plants due to their ability to cause changes in the nucleotide sequence.

Tomato plants improve through breeding for agronomic traits such as higher fruit yield, and resistance to pests and diseases basically because of low genetic diversity. Therefore, in this study the already developed mutant population of tomato cobra variety using induced mutation with EMS was evaluated and traits such as fruit yields and ability to resist blossom end rot disease were noted. The variants population selected in this study will be useful in identifying novel genes for tomato breeding and improvement as well as being a platform for further genetics studies.

MATERIALS AND METHODS

The experiment was conducted at the Teaching and Research Farm of the Department of Crop Science, University of Nigeria, Nsukka, Enugu State Nigeria. The site is located on latitude 06º 53′ N, longitude 07º 24′ E, and altitude of 447.30 m asl. The rainfall is bimodal with an annual total of about 1,500 mm. The relative humidity ranges from 70 to 80%, and the ambient temperature from 20 to 30°C during the rainy season (Manggoel and Uguru, 2011). The tomato variety used was a popular market brand cobra, which is highly cherished by consumers but has poor performance in the field due to excessive precipitation. The mutagenic agent used is ethyl methane sulfonate (EMS). A stock solution of EMS was prepared using distilled water. The stock solution

Please cite as: Ilo G.E., Uguru M.I., Ogbonna P.E. and Amuji C.F. (2023). Field evaluation of M4 plants obtained from induced mutation of tomato 'cobra' variety with ethyl methane sulfonate (EMS). *Agro-Science*, **22 (2),** 73-77. DOI: https://dx.doi.org/10.4314/as.v22i2.10

was then used to prepare, 0.01%, 0.1%, 0.25%, and 0.5% EMS concentrations using 0.1 M phosphate buffer (pH 7.2). These concentrations were used to induce mutation on the cobra tomato variety. In the M1 generation, ripe fruits were harvested and packed according to treatment and used in the M2 generation. In the M2 and M3 generations, data on qualitative and quantitative traits were taken and selections based on the performance of the variants in the field. Variants that performed optimally in terms of yield, earliness to flowering and maturity and their tolerance level to blossom end rot disorder formed the basis for this selection.

Seven selected variants and the untreated plant from the M3 generation were evaluated in the field as M4 for this particular study. Seeds of selected M3 variants and the untreated seed were pre-soaked in water and then planted in Petri dishes to enhance germination. The sprouted seeds were planted in plastic bags in the nursery. The seedling was raised in the nursery growth medium of compost in plastic bags. Three seedlings were planted in a bag and later thinned to two per bag. Watering was done when necessary until they mature for transplanting. The seedlings spent three to four weeks in the nursery before transplanting them to the field.

The experimental field was ploughed, harrowed, and ridged. The experiment was laid out in a randomized complete block design (RCBD) with three replications. Seedlings were planted at a spacing of 1 m between ridges and 0.45 m within ridges. Poultry manure was applied at the rate of 0.3 tonnes on a field of 300 m^2 to the ridges to enhance the nutrient capacity of the soil. Phyto-feed was also applied at the same rate in a ring form two weeks after transplanting to enhance the soil nutrients. Weeding was carried out manually using hoes at regular intervals to ensure a weed-free field.

Characterization of qualitative and quantitative traits was done following the International Board for Plant Genetic Resources (IPGR/IITA/BAMNET, 1995) descriptors for tomatoes. Qualitative data collected are fruit size at maturity, fruit shape, petiole colour, branching habit, fruit structure at maturity, fruit colour before ripening, and colour when ripened. Quantitative data collected are days to 50% flowering and days to 50% maturity. number of flowers per truss, fruit weight per plant, number of fruits per plant and number of fruits harvested per plant, number of flowers per truss, number of leaves, leaf length, leaf width, number of branches, number of fruits per truss, number of trusses per plant, number of fruits affected by blossom end rot, plant girth, and plant height.

Analysis of variance was carried out on the data, and significant means were separated using F-LSD at a 5% probability level. Also, genotypic variance, phenotypic variance and heritability estimate were checked according to Singh and Choudhary (1985). Genotypic variance (σ^2g) using the formula:

$$PCV = \sigma^2pX \times 100, GCV = \sigma^2gX \times 100;$$

where MSG is mean square of genotypes, MSE is mean square of error, and *r* is number of replications. Phenotypic variance (σ^2p) was obtained as:

$$\sigma^2p = \sigma^2g + \sigma^2e;$$

where σ^2g is genotypic variance, and σ^2e is mean squares of error. Others are:

$$\text{Error variance } (\sigma^2e) = MSE,$$
$$\text{genotypic variance} = \sigma^2gX \times 100,$$
$$\text{phenotypic variance} = \sigma^2pX \times 100;$$

where σ^2g is genotypic variance, σ^2p is phenotypic variance, *X* is mean of the trait. Then, broad sense heritability estimate ($H^2\%$) was obtained as:

$$H^2\% = \sigma^2g \times \sigma^2p \times 100.$$

In this particular analysis, the $H^2\%$ was categorized by Robinson *et al.* (1949) as low (0-30%), moderate (30-60%) and high (≥ 60). Expected and estimated genetic advance (GA) was calculated as:

$$GA = k\sigma pH^2;$$

where the selection intensity (*k*) is 5%, *k* (a constant) is 2.06, σp is phenotypic standard deviation, H^2 is the broad sense heritability. The genetic advance as percentage of mean (GA) = $k\sigma pH^2$; this GA was categorized as low (0-10%), moderate (10-20%), and high (> 20%) (Johnson *et al.,* 1955).

RESULTS AND DISCUSSION

In Figure 1, the average mean of the leaf length and width had a significant effect among the variants. The longest and broadest leaves were recorded by the untreated plant (CP1) while the shortest was recorded by variant 8. The mean number of branches per plant had a significant effect among the variants with the highest number of branches at flower bud initiation being recorded by variant 4 while variant 2 had the least. Variants 3, 5, 6 and 8 were similar. The highest mean number of leaves per plant was recorded by variant 6 which were significantly different from the least recorded by variant 2. There was a significant difference on the mean number of truss per plant with the highest number recorded by variant 3 while the untreated recorded the least. Variants 2, 4 and 5 were similar. Variant 3 had the highest plant girth which was significantly different from the untreated plant with the least mean plant girth. The other variants studied were similar. The highest mean plant height was recorded by the untreated plant while variant 5 had the least. The other variants were similar (Figure 1). The plant heights of variants were affected by the mutagen treatment in this experiment and variability was observed in the tomatoes. Other researchers found similar results of variabilities in plant height due to mutagen treatment (Okuno and Kawait, 1978; Jabeen and Mirza, 2002). However, plants possessing desired traits were selected in this study.

As shown in Table 1, the mean number of days to 50% flowering had a significant difference on the variants studied. The shortest number of days was recorded by variant 3 (42.2), while the longest

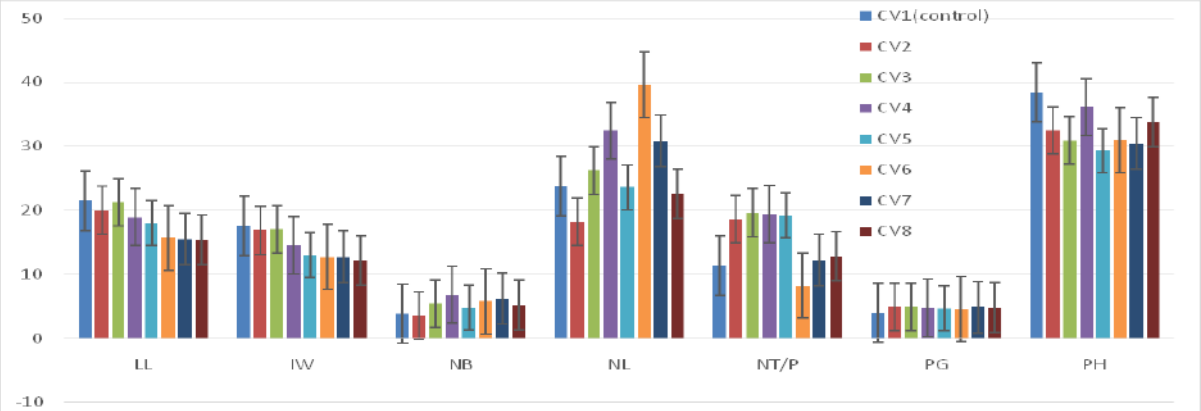

Figure 1: Mean of the leaf length, leaf width, number of branches, number of truss per plant, plant height, and plant girth of the evaluated eight variants of the M4 cobra tomato variety
LL - leaf length, LW - leaf width, NB - number of branches, NL - number of leaves per plant,
NT/P - number of truss per plant, PG - plant girth, PH - plant height

Table 1: Number of days to 50% flowering, number of days to maturity, fruit weight per plant, number of fruits harvested, number of flowers per truss, number of fruits per truss and number of fruits affected by blossom end rot of eight M4 variants of cobra tomatoes variety

Mutants	D50%F	DM	FW/P	NFH	NFL/T	NFR/P	NFR/T	NBER
Cp1 (control)	48.20	82.40	479.00	14.80	5.60	19.20	2.80	3.60
CV2	43.00	77.00	1069.00	17.00	6.40	24.80	3.80	0.00
CV3	42.20	74.40	1118.00	23.60	6.60	37.00	4.00	0.00
CV4	43.00	75.00	956.00	18.40	6.40	27.20	4.20	0.00
CV5	45.40	75.00	1078.00	22.60	6.00	33.80	3.20	0.00
CV6	45.00	75.80	412.00	13.20	4.80	11.00	2.20	0.00
CV7	48.60	82.80	318.00	8.60	5.80	14.60	3.60	0.00
CV8	49.20	84.20	574.00	21.20	5.40	21.60	3.20	0.00
F-LSD ($p < 0.05$)	2.312	2.401	440.600	8.990	1.354	12.068	1.236	0.251

D50%F - days to 50% flowering, DM - days to maturity, FW/P - fruit weight per plant, NFH - number of fruits harvested per plant,
NFL/T - number of flowers per truss, NFR/P - number of fruits per plant, NFR/T - number of fruits per truss,
NBER - number of fruits affected by blossom end rot

number of days to 50% flowering was recorded by variant 8 (49.2). The Mean number of days to 50% flowering of variants 2, 4, 5 and 6 were statistically similar. There was a significant effect on the number of days to maturity with the lowest number of days to fruit maturity recorded by variant 3 (74.4), followed closely by variants 4 (75), 5 (75), and 6 (75.8). The longest number of days to fruit maturity was recorded by the untreated plant (84.4). The highest number of flowers per truss was recorded by variant 3 which were significantly different from the lowest recorded by variant 6. The number of fruits harvested per plant had a significant effect among the variants with variant 3 (23.6) recording the highest number of fruits. This was followed by variants 5 (22.6) and 8 (21.2). The lowest number of fruits harvested was recorded by variant 7. The number of fruits per truss equally had significant effect on the variants. The highest number was recorded by variant 4 while the least was recorded by variant 6. The mean fruit weight per plant had a significant effect with the highest fruit weight per plant being recorded by variant 3 (1.118 kg), followed closely by variants 5 (1.078 kg), and 2 (1.069 kg). The least weight was recorded by the untreated (318 g). The highest number of fruits affected by blossom end rot was recorded by the untreated plant which was significantly higher than the rest of the variants studied (Table 1).

In this M4 generation, seven variants selected from the M3 generation were evaluated in the field along with the parent plant as M4 plants. Variant 3 had an early flowering trait attaining 50% flowering at 42 days from the planting date as against the parent plant that attains 50% flowering at 48 days. The same variant matures earlier than all the other variants studied. The same variant yields higher than the others studied in this study. The mean fruit weight recorded by this variant was 1.118 kg with 23.6 numbers of fruits harvested per plant as against the parent plant that recorded 479 g and 14 fruits actually harvested per plant. The size of a single fruit for variant 3 was 47 g, while the parent plant produced smaller fruits each weighing 34 g. In this M4 generation of cobra, however, there was a zero attack on all the variants studied as compared to the number of the parent plants (3.6) attacked by the blossom end rot disorder.

Furthermore, in this study, to achieve improvement of yield in the test crop (cobra tomato variety), the extent of variation existing in the gene pool was determined using heritability and genetic spread. This approach is in accordance with previous report that for improvement to be achieved of a specific plant character, it must not only depend on available genetic variation but also the extent of heritability of such variation (Umar *et al.,* 2014). Previous work

Table 2: Estimation of genetic parameters of selected agronomic and yield traits of cobra tomato variety

Trait	σ^2g	σ^2e	σ^2p	GCV	PCV	H^2%	GA (%)
Days to 50% flowering	18.77	1.82	20.59	56.06	58.42	91.00	8.50
Days to maturity	14.23	3.08	17.31	38.45	42.40	82.00	7.02
Fruit weight per plant	37.39	12.61	50.00	161.00	187.05	74.00	10.77
Number of flowers per truss	0.37	33.15	1.40	24.29	42.23	26.00	0.63
Number of fruits harvested per plant	64.09	33.15	97.24	155.81	191.97	66.00	13.40
Number of fruits per plant	55.46	42.97	98.43	132.69	176.77	56.00	11.45
Number of fruits per truss	0.32	0.45	0.77	29.13	45.19	41.00	0.74
Number of fruits affected by blossom end rot	40.52	28.47	68.99	397.07	518.12	59.00	6.30
Leaf length	4.78	3.27	8.05	51.56	67.06	59.00	3.45
leaf width	23.74	34.87	58.61	132.00	207.26	41.00	6.47
Number of branches	0.99	1.07	2.06	44.36	63.96	48.00	1.42
Number of leaves	20.16	34.85	55.01	90.71	149.84	37.00	5.65
Number of truss per plant	8.66	22.40	31.06	72.82	137.91	28.00	3.22
Plant girth	0.18	0.18	0.36	19.08	27.14	49.00	0.61
Plant height	8.51	6.89	15.40	52.93	52.93	55.00	4.45

σ^2g - genotypic variance, σ^2p - phenotypic variance, σ^2e - environmental variance, GCV - genetic coefficient of variation, PCV - phenotypic coefficient of variation, GA - genetic advance, H^2% - heritability

equally noted that the estimates of heritability together with genetic advance provide profound advantage over sole of heritability (Akhtar *et al.,* 2007; Jandong *et al.,* 2020; Yoseph *et al.,* 2022).

Estimates of Variance Components, Heritability and Genetic Advance

The choice of selection method specified for the improvement of yield in tomatoes was based on the measure of the amount of variation that exists in the gene pool of the crop, estimates of heritability, and genetic advance (Table 2). This is in line with the report of previous works on heritability, which observed that the selection made for the improvement of a character is not only dependent on available genetic variation but also on the extent of heritability of such variations (Umar *et al.*, 2014). Previous work equally noted that the estimates of heritability together with genetic advances provide a profound advantage over the use of heritability alone (Shukla *et al*., 2006; Asfaw *et al.,* 2017). From the results of this study, a higher PCV than GCV indicates a slight influence of the environment on the traits and there was the possibility of improvement using phenotypic selection. The result of this work was in line with the report of previous works by (Ashok *et al.,* 2000; Uguru 2000; Adebola *et al*., 2002).

However, the high GCV in most traits studied shows that there was little influence of the environmental changes which guarantees selection for such traits. This was in agreement with the work of Hefny (2011) who submitted that high GCV estimates are indicative of low amenability of traits to environmental changes. Typically, characters with reasonable variation offer a wide range of opportunities for selection for their improvement (Fakuta *et al*., 2014). However, characters with low GCV and PCV values showed low variability among the variants with lesser opportunity for selection and crop improvement. Johnson *et al.* (1955) classified heritability estimates as low for 0-30%, moderate for 30-60% and high for values above 60%.

CONCLUSION AND RECOMMENDATIONS

The study has produced lots of variants as a result of induced mutation with the best yield got from most of the variants as compared to the parent plant. High yielding variants 3 and 5 with fruit yields of 1.118 and 1.069 kg, respectively with zero incidence of blossom end rot disorder is recommended.

AUTHORS' CONTRIBUTIONS

Conceptualization of research (IGE); designing of the experiments (IGE, UMI, OPE); contribution of experimental materials (IGE, UMI, OPE, ACF); execution of field/lab experiments and data collection (IGE); analysis of data and interpretation (IGE and ACF); preparation of manuscript (IGE, UMI, OPE and ACF).

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