International Journal of Plant & Soil Science

Volume 37, Issue 9, Page 182-195, 2025; Article no.IJPSS.142764
ISSN: 2320-7035

Genetic Diversity and Drought Tolerance in M7 Ethidium Bromide-Derived Tomato Genotypes

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: https://doi.org/10.9734/ijpss/2025/v37i95697

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://pr.sdiarticle5.com/review-history/142764

Original Research Article

Received: 21/06/2025
Published: 26/08/2025

ABSTRACT

This study evaluated the extent of genetic variability for drought tolerance, as well as the interplay among key drought-tolerant traits of M7 ethidium bromide (EtBr)-derived tomato genotypes, under polyethylene glycol (PEG)-induced drought stress. The study assessed ten of tomato genotypes under varying PEG concentrations (0%, 5%, and 10%) using a Completely Randomized Design. Statistical analysis was conducted on data including germination capacity and energy, root length, root count, shoot length, and both fresh and dry shoot weight. ANOVA revealed significant variability among genotypes for all traits. Higher concentrations of PEG led to reduced germination and weaker seedling performance. However, genotypes G3, G8, G9, and G10 exhibited superior drought tolerance, as reflected in their extensive root development. Based on Stress Tolerance

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Cite as: *Abiola Toyin Ajayi, Oloruntoba Emmanuel Oladipo, Daniel Adegunle Adelakin, and Maleeq Babatunde Badmus. 2025. "Genetic Diversity and Drought Tolerance in M7 Ethidium Bromide-Derived Tomato Genotypes". International Journal of Plant & Soil Science 37 (9):182–195. https://doi.org/10.9734/ijpss/2025/v37i95697.*

Indices (STIs), genotypes G2, G3, G8, G9, and G10 were effectively ranked as highly drought-tolerant. High phenotypic coefficient of variation (PCV), genotypic coefficient of variation (GCV) (>20%), and heritability (>60%) were observed for germination energy and capacity, root count, and fresh shoot weight. Consistent correlations were observed between germination capacity and traits such as shoot height and root length. The study reveals substantial genetic variability among EtBr-derived tomato genotypes, with G3, G8, G9, and G10 exhibiting notable drought tolerance. High heritability and strong inter-trait correlations underscore the breeding potential of these genotypes for developing drought-resistant tomato cultivars.

Keywords: Drought tolerance; ethidium bromide; germination capacity; mutagenesis; tomato.

1. INTRODUCTION

Tomato is a popular and economically important vegetable species worldwide due to its Antioxidative and anticancerus properties (Parveen et al., 2019; Jogi et al., 2023). In Nigeria, tomato production is notably lower at 4.1 million tons compared to Egypt's production of 7.39 million tons (Dube et al. 2020). This disparity may be attributed to various environmental stresses impacting productivity. Among the environmental stresses, drought is one of the major limiting factors of tomato fruit growth and production (Musa and Kolawole, 2024).

Drought stress is a significant environmental factor that profoundly impacts plant growth, development, and productivity. Plants experience drought stress when an inadequate supply of water is available for normal physiological processes, leading to various physiological, chemical, and molecular changes in plant cells and tissues (Farooq et al., 2009). The key effects of drought stress is the disruption of water balances within plant cells, resulting in cellular dehydration and osmosis (Chaves et al., 2009). Dehydration can reduce cell turgor pressure, affecting cell expansion, leaf morphology, and overall plant growth (Flexas and Mendrano, 2002). Additionally, prolonged drought triggers a series of biochemical responses in plants, including the accumulation of reactive oxygen species (ROS) such as superoxide radicals, hydrogen peroxide, and hydroxyl radicals (Mittler, 2002). ROS accumulation can cause damage to cellular components such as lipids, proteins, and nucleic acids, leading to cellular dysfunction and even cell death (Apel and Hirt, 2004). Drought stress influences plant hormone signaling pathways, particularly abscisic acid (ABA), which is central in regulating stomatal closure to reduce water loss through transpiration (Fujita et al., 2005).

Plants respond and adapt to, and yet to survive under drought stress by the induction of various morphological, biochemical, and physiological responses (Beck et al., 2007). Physiologically, plants respond to water scarcity by regulating the stomatal aperture to minimize water loss through transpiration (Flexas and Medrano, 2002), engaging in osmotic adjustment through the accumulation of compatible solutes like proline and sugars to maintain cellular turgor (Farooq et al., 2009), and optimizing water and retention through root systems and cuticular transpiration central (Chaves et al., 2009). At the molecular level, plants orchestrate responses through signaling molecules like abscisic acid, triggering stomatal closure and activating stress-responsive gene expression pathways (Fujita et al., 2005). They also bolster cellular stress through osmoprotectants and antioxidant enzymes such as superoxide dismutase (SOD) and catalase (CAT) (Mittler, 2002).

Over time, diverse methods have been utilized to identify drought-tolerant genotypes, with previous efforts focusing on screening tomato varieties exhibiting varying levels of drought tolerance (Dias, 2010; 2014). The utilization of polyethylene glycol (PEG) in *in vitro* selection techniques stands as a dependable method for screening desirable genotypes and further investigating the impact of water scarcity on plant germination indices (Sakthivelu et al., 2008). PEG, encompassing a range of polymers from viscous liquids to waxy solids, has been used to artificially induce water stress in plants (George et al., 2015). Being inert, non-ionic, and virtually impermeable to cell membranes, PEG can uniformly induce water stress without causing direct physiological damage to plants (Ghebremariam et al., 2013). Research indicates that PEG-induced osmotic stress leads to a reduction in cell water potential (Govindaraj et al., 2010). Khodarahmpour (2011) observed that an increase in the concentration of PEG-6000 led to a decrease in germination rate, root length, shoot length, and seed vigour in specific plants.

Despite the evident impact of water stress on crop yields, there has been limited research on drought resistance in Nigeria, particularly concerning tomato cultivation. Tomato genotypes typically exhibit limited genetic variability for drought tolerance. Consequently, one of the most effective approaches to mitigate the effects of drought stress involves inducing mutations in cultivated tomato varieties to create drought-tolerant lines (Hallajian, 2016). Hence, the present study aims to evaluate M7 ethidium bromide (EtBr)-derived tomato genotypes for drought tolerance and inter-character association under PEG-induced drought stress.

2. MATERIALS AND METHODS

2.1 Location of the Study

The study was conducted at the Experimental Laboratory of the Department of Plant Science and Biotechnology, Adekunle Ajasin University, Akungba-Akoko, between November and December 2023.

2.2 Plant Materials

Ten tomato genotypes were collected from the Plant Breeding Unit of the Department of Plant Science and Biotechnology. These genotypes, derived from ethidium bromide (EtBr) treatments, were at the M7 generation (Table 1). Initially, seeds from two tomato accessions received from the National Centre for Genetic Resources and Biotechnology (NACGRAB), Nigeria, were treated with 0.5% (v/v) EtBr for 0, 24, 48, 72, and 96 hours. The seeds were germinated in baskets, and the seedlings were later transplanted into the field, where they were raised from the M1 to M6 generations.

2.3 Methodology

The assessment of drought tolerance among the tomato genotypes was carried out in the laboratory, following the methodology outlined by Shamim et al. (2014) with some modifications. The seeds were surface sterilized using 10% sodium hypochlorite (NaClO) solution for 1 hour, followed by five rinses with distilled water. Afterward, the seeds were planted in sterilized plastic containers measuring 15 cm in length, 9 cm in width, and 5 cm in height. Each container contained two pieces of sterilized filter paper and 5 ml of polyethylene glycol (PEG) at three concentration levels (0%, 5%, and 10%). Within each plastic container, twenty seeds of each genotype were positioned on the filter papers, and this experimental setup was replicated three times for each treatment, employing a Completely Randomized Design (CRD). Subsequently, the plastic containers were placed in a dark room at room temperature for an initial incubation period of 48 hours. Following the initial incubation, the photoperiod was adjusted to 12 hours of light and 12 hours of darkness at room temperature. The experiment concluded at the seedling stage, occurring 28 days after planting.

2.4 Data Collection and Analysis

Data were collected on germination capacity and energy, root length, number of roots, shoot length, fresh and dry shoot weight, and fresh and dry root weight. Data were subjected to analysis of variance, and the means were separated with Duncan's Multiple Range Test (DMRT) at a $P \leq 0.05$ significance level using SPSS version 20. Drought tolerance indices for all traits were computed using the Stress Tolerance Index (STI), following the method of Ajayi (2021): $STI = \frac{C \times S}{\bar{C}^2}$ where *C* is the mean trait value of a genotype under control conditions, *S* is the mean under stress, and $\bar{C}$ is the grand mean across all genotypes under control conditions. Genotypes were ranked into drought tolerance classes from 1 (most tolerant) to 10 (least tolerant) for each trait. Genetic parameters were estimated as described by Ajayi et al. (2024): Error variance (V_E) = Mean square error (MS_E). Genotype × treatment variance (V_{GT}) = $\sigma^2_{GT} = (MS_{GT} - MS_E)/r$. Genotypic variance ($V_G$) = $(MS_G - MS_E)/rT$. Phenotypic variance (V_P) = $V_G + V_{GT}/T + V_E/rT$. Genotypic coefficient of variation (*GCV*) = $\frac{\sqrt{V_G}}{\bar{X}} \times 100$. Phenotypic coefficient of variation (*PCV*) = $\frac{\sqrt{V_P}}{\bar{X}} \times 100$. Broad-sense heritability (H^2) = $V_G/(V_G + (V_{GT}/T) + (V_E/rT))$. Genetic advance (GA) = $\frac{VG}{\sqrt{VP}} \times k$; k = 2.06 (selection differential). Genetic advance as percent of the mean (GAM) = $\frac{GA}{\bar{\chi}} \times 100$; where T, $\bar{X}$, and r are the number of treatments, grand mean of trait, and replicates, respectively. Estimates of genetic parameters were categorized as cited in Ajayi et al. (2014). Pearson's correlation analysis among germination and seedling traits was estimated with SPSS at $P \leq 0.05$ and 0.01 significance levels.

Table 1. List of genotypes evaluated for drought tolerance under varying peg-induced stress conditions

S/N	Genotype code	Accession ID	EtBr Treatment (0.5%)
1	G1	NG/AA/SEP/09/042	0 hour
2	G2	NG/AA/SEP/09/042-EtBr24	24 hours
3	G3	NG/AA/SEP/09/042-EtBr48	48 hours
4	G4	NG/AA/SEP/09/042-EtBr72	72 hours
5	G5	NG/AA/SEP/09/042-EtBr-96	96 hours
6	G6	AKUNGBA2	0 hour
7	G7	AKUNGBA2-EtBr24	24 hours
8	G8	AKUNGBA2-EtBr48	48 hours
9	G9	AKUNGBA2-EtBr72	72 hours
10	G10	AKUNGBA2EtBr96	96 hours

3. RESULTS AND DISCUSSION

The ANOVA results in Table 2 revealed that the effects of genotype, treatment, and the genotype × treatment interaction were significant for all the studied traits. Among the traits, germination energy exhibited the highest coefficient of variation (CV) at 30%, while shoot height showed the lowest CV (7%).

3.1 Mean Performance for Germination and Seedling Traits among EtBr-Derived Tomato Genotypes Evaluated for Drought Tolerance under Varying PEG-induced Stress Conditions

The mean performance for germination and seedling traits among EtBr-derived tomato genotypes evaluated for drought tolerance under different PEG treatments is presented in Tables 3 to 5. Under control conditions (Table 3), the highest mean germination energy value (25%) was observed in G5 and G7, while the lowest (1.67%) was observed in G1. For germination capacity, G7 had the highest value (50%) and G6 had the lowest (5%). Shoot height varied from 3.03 cm in G2 to 5.87 cm in G5. Root length ranged from 1.91 cm in G10 to 4.55 cm in G1. The number of roots ranged from 1.91 in G10 to 4.55 in G1. Fresh shoot weight was highest (0.004 g) in G2, G4, and G7 and lowest (0.02 g) in G6. Dry shoot weight ranged from 0.0005 g in G3 to 0.004 g in G4.

Under 5% PEG treatment (Table 4), germination energy peaked at 18.33% in G7 and dropped to 1.67% in G1. G9 exhibited the highest germination capacity (36.67%), whereas G6 had the lowest (5%). Shoot height ranged from 1.00 cm in G6 to 4.60 cm in G10. Root length varied from 2.88 cm in G7 to 0.88 cm in G5. The number of roots ranged from 1.00 in G6 to 3.30 in G9. Fresh shoot weight was highest (0.30 g) in G1 and G3, and lowest (0.20 g) in G2 and G4 through G10. The highest dry shoot weight (0.003 g) was observed in G1, G3, G4, G5, and G9, while G6 had the lowest value (0.0003 g).

Under 10% PEG treatment (Table 5), no germination energy was recorded for G5, G6, and G7 across all traits. Germination capacity reached its highest value (20%) in G3 and G9, and its lowest (10%) in G1 and G2. Shoot height varied from 2.18 cm in G4 to 0.90 cm in G3. Root length ranged from 0.30 cm in G4 to 2.86 cm in G3. The number of roots was highest (2.00) in G2 and lowest (1.33) in G1, G8, and G10. Fresh shoot weight ranged from 0.001 g in G1 to 0.02 g in G3, G9, and G10. For dry shoot weight, the highest value (0.003 g) was observed in G3, and the lowest (0.001 g) in G4.

Table 2. Combined mean square values for genotype, treatment, and genotype × treatment interaction among EtBr-derived tomato genotypes evaluated for drought tolerance under varying PEG-induced stress conditions

Source of variation	DF	GE (%)	GC (%)	SH (cm)	RL (cm)	NR	FSW (g)	DSW (g)
Genotype (G)	9	241.64*	690.86*	2.76*	1.96*	4.16*	0.0002*	0.000024*
Treatment (T)	2	1025.28*	2167.78*	105.01*	5.08*	19.45*	0.004*	0.000012*
G × T	18	84.238*	222.72*	2.59*	1.27*	1.81*	0.0001*	0.00004*
Error	60	29.72	70.28	0.42	0.13	0.47	0.000028	0.000002
Coefficient of variation		30.00	15.43	7.00	8.54	10.31	8.82	23.57

**: Significant at P≤0.05; ns: not significant. DF: Degree of freedom. GE: Germination energy; GC: Germination capacity; SH: Shoot height; RL: Root length; NR: Number of roots; FSW: Fresh shoot weight; DSW: Dry shoot weight.*

Table 3. Mean performance for germination and seedling traits among EtBr-derived tomato genotypes evaluated for drought tolerance under control conditions

Genotype	GE (%)	GC (%)	SH (cm)	RL (cm)	NR	FSW (g)	DSW (g)
G1	1.67±1.67^{a}	20.00±2.89^{ab}	4.87±0.31^{bc}	1.70±0.22^{bc}	4.55±0.66^{d}	0.03±0.005^{ab}	0.001±0.0001^{a}
G2	3.33±1.67^{a}	13.33±1.67^{ab}	3.03±0.73^{a}	1.16±0.29^{ab}	2.55±0.29^{abc}	0.04±0.01^{ab}	0.001±0.0001^{a}
G3	5.00±2.89^{a}	18.33±6.01^{ab}	4.14±0.69^{ab}	1.46±0.05^{abc}	2.25±0.29^{ab}	0.03±0.002^{ab}	0.0005±0.0001^{a}
G4	11.67±4.41^{a}	21.67±6.01^{b}	5.10±0.15^{bc}	1.67±0.18^{bc}	2.25±0.29^{ab}	0.04±0.003^{ab}	0.004±0.001^{b}
G5	25.00±7.64^{b}	38.33±7.27^{c}	5.87±0.36^{c}	1.82±0.12^{c}	2.80±0.0^{abc}	0,04±0.003^{b}	0.003±0.0002^{b}
G6	1.67±1.67^{a}	5.00±2.89^{a}	4.60±0.30^{bc}	1.03±0.13^{a}	2.33±0.67^{ab}	0.02±0.006^{a}	0.002±0.001^{a}
G7	25.00±5.00^{b}	50.00±5.78^{d}	5.23±0.28^{bc}	1.49±0.04^{abc}	3.93±0.29^{cd}	0.04±0.0006^{ab}	0.001±0.0001^{a}
G8	13.33±1.67^{ab}	28.33±1.67^{bc}	5.22±0.22^{bc}	1.38±0.04^{abc}	2.75±0.38^{abc}	0.03±0.004^{ab}	0.002±0.001^{a}
G9	25.00±5.00^{b}	48.33±1.67^{d}	5.21±0.17^{bc}	1.79±0.10^{c}	3.60±0.64^{bcd}	0.03±0.006^{ab}	0.001±0.0001^{a}
G10	5.00±2.89^{a}	23.33±7.27^{bc}	4.56±0.57^{bc}	1.20±0.24^{ab}	1.91±0.30^{a}	0.03±0.005^{ab}	0.001±0.0001^{a}
GM	11.67±2.04	26.67±2.89	4.78±0.18	1.48±0.06	2.89±0.19	0.03±0.001	0.001±0.0002

Mean values followed by similar superscripts within a column are not significantly different from one another at P≤0.05 using DMRT. GM: Grand mean; GE: Germination energy; GC: Germination capacity; SH: Shoot height; RL: Root length; NR: Number of roots; FSW: Fresh shoot weight; DSW: Dry shoot weight.

Table 4. Mean performance for germination and seedling traits among EtBr-derived tomato genotypes evaluated for drought tolerance under 5% PEG treatment

Genotype	GE (%)	GC (%)	SH (cm)	RL (cm)	NR	FSW (g)	DSW (g)
G1	1.67±1.67^{a}	15.00±5.00^{abc}	4.35±2.35^{ef}	1.60±0.29^{bc}	2.67±0.33^{bc}	0.30±0.001^{c}	0.003±0.0003^{c}
G2	3.33±1.67^{ab}	10.00±2.89^{ab}	2.99±0.14^{bc}	1.35±0.37^{ab}	3.17±0.88^{bc}	0.02±0.002^{b}	0.002±0.0002^{a}
G3	3.33±1.67^{ab}	15.00±7.64^{abc}	3.04±0.19^{bcd}	2.39±0.29^{de}	1.78±0.29^{ab}	0.03±0.004^{c}	0.003±0.00003^{bc}
G4	5.00±2.89^{ab}	10.00±2.89^{ab}	2.18±0.12^{b}	1.08±0.18^{ab}	2.00±0.29^{ab}	0.02±0.0003^{a}	0.003±0.00003^{c}
G5	6.67±4.41^{abc}	13.33±6.01^{ab}	3.33±0.36^{cde}	0.85±0.08^{a}	2.67±0.30^{bc}	0.02±0.0^{a}	0.003±0.0001^{bc}
G6	0.0±0.0^{a}	5.00±0.0^{a}	1.00±0.0^{a}	1.60±0.0^{bc}	1.00±0.0^{a}	0.02±0.0^{a}	0.0003±0.0^{bc}
G7	18.33±8.82^{c}	31.67±12.02^{bc}	4.04±0.20^{def}	2.88±0.24^{e}	2.47±0.18^{bc}	0.02±0.0^{a}	0.002±0.0001^{a}
G8	10.00±5.00^{abc}	21.67±7.27^{abc}	3.79±0.62^{cdef}	2.38±0.20^{de}	2.50±0.0^{bc}	0.02±0.0^{a}	0.002±0.0001^{a}
G9	15.00±0.0^{bc}	36.67±6.01^{c}	4.53±0.32^{f}	2.06±0.25^{cd}	3.33±0.63^{c}	0.02±0.0^{a}	0.003±0.0002^{a}
G10	1.67±1.67^{a}	21.67±9.28^{abc}	4.60±0.30^{f}	1.60±0.11^{bc}	2.75±0.38^{bc}	0.02±0.0^{a}	0.002±0.0003^{a}
GM	6.50±1.46	18.00±2.50	3.39±0.22	1.78±0.12	2.43±0.16	0.02±0.001	0.003±0.001

Mean values followed by similar superscripts within a column are not significantly different from one another at P≤0.05 using DMRT. GM: Grand mean; GE: Germination energy; GC: Germination capacity; SH: Shoot height; RL: Root length; NR: Number of roots; FSW: Fresh shoot weight; DSW: Dry shoot weight.

Table 5. Mean performance for germination and seedling traits among EtBr-derived tomato genotypes evaluated for drought tolerance under 10% PEG treatment

Genotype	GE (%)	GC (%)	SH (cm)	RL (cm)	NR	FSW (g)	DSW (g)
G1	0.0±0.0	10.00±0.0^{c}	1.07±0.52^{abc}	0.63±0.14^{b}	1.33±0.33^{b}	0.001±0.001^{b}	0.003±0.001^{bc}
G2	0.0±0.0	10.00±0.01^{c}	2.17±1.01^{c}	0.77±0.22^{bc}	2.00±0.58^{b}	0.01±0.003^{cd}	0.002±0.0001^{bc}
G3	0.0±0.0	20.00±0.0^{e}	2.18±0.09^{c}	2.86±0.24^{f}	1.78±0.11^{b}	0.02±0.002^{de}	0.003±0.0003^{bc}
G4	0.0±0.0	6.67±0.33^{b}	0.90±0.0^{ab}	0.30±0.0^{ab}	1.50±0.29^{b}	0.01±0.001^{bc}	0.001±0.0001ab
G5	0.0±0.0	0.0±0.0^{a}	0.0±0.0^{a}	0.0±0.0^{a}	0.0±0.0^{a}	0.0±0.0^{a}	0.0±0.0^{a}
G6	0.0±0.0	0.0±0.0^{a}	0.0±0.0^{a}	0.0±0.0^{a}	0.0±0.0^{a}	0.0±0.0^{a}	0.0±0.0^{a}
G7	0.0±0.0	0.0±0.0^{a}	0.0±0.0^{a}	0.0±0.0^{a}	0.0±0.0^{a}	0.0±0.0^{a}	0.0±0.0^{a}
G8	0.0±0.0	15.00±0.0^{d}	1.43±0.18^{bc}	2.30±0.10^{e}	1.33±0.17^{b}	0.01±0.002^{bc}	0.003±0.0003^{bc}
G9	0.0±0.0	20.00±0.0^{e}	1.85±0.23^{bc}	1.30±0.37^{cd}	3.99±0.67^{c}	0.02±0.001^{e}	0.003±0.001^{c}
G10	0.0±0.0	15.00±0.0^{d}	1.18±0.09^{abc}	1.48±0.27^{d}	1.33±0.33^{b}	0.02±0.001^{d}	0.003±0.0003^{c}
GM	0.0±0.0	9.67±1.42	1.08±0.18	0.96±0.18	1.33±0.23	0.01±0.001	0.002±0.0002

Mean values followed by similar subscripts within a column are not significantly different from one another at $P \leq 0.05$ using DMT. GM: Grand mean; GE: Germination energy; GC: Germination capacity; SH: Shoot height; RL: Root length; NR: Number of roots; FSW: Fresh shoot weight; DSW: Dry shoot weight.

Table 6. Stress tolerance indices (STIs) for germination and seedling traits and (ranking) of EtBr-derived tomato genotypes evaluated for drought tolerance under varying PEG-induced stress conditions

5% PEG Treatment										
Genotype	**GE (%)**	**GC (%)**	**SH (cm)**	**RL (cm)**	**NR**	**FSW (g)**	**DSW (g)**	**RkSum**	**RkM**	**RkSD**
G1	0.00 (8)	0.80 (5)	0.89 (3)	0.94 (8)	0.59 (9)	0.10 (10)	0.30 (3)	46	6.57	2.88
G2	0.00 (8)	0.80 (6)	0.99 (2)	1.17 (6)	1.24 (2)	0.50 (3)	0.30 (3)	30	4.29	2.36
G3	0.67 (3)	0.80 (4)	0.73 (6)	1.64 (3)	0.79 (7)	1.00 (1)	0.60 (2)	26	3.71	2.14
G4	0.43 (5)	0.50 (8)	0.43 (9)	0.64 (9)	0.89 (6)	0.50 (3)	0.75 (1)	41	5.86	3.08
G5	0.27 (7)	0.40 (9)	0.56 (8)	0.47 (10)	0.95 (3)	0.50 (3)	0.10 (6)	46	6.57	2.76
G6	0.00 (8)	0.00 (10)	0.22 (10)	1.55 (4)	0.43 (10)	1.00 (1)	0.15 (5)	48	6.86	3.58
G7	0.73 (2)	0.60 (7)	0.77 (10)	1.93 (1)	0.63 (8)	0.50 (3)	0.20 (4)	35	5.00	3.37
G8	0.75 (1)	0.80 (3)	0.72 (5)	1.73 (2)	0.91 (5)	0.67 (2)	0.10 (6)	24	3.43	1.90
G9	0.60 (4)	0.80 (2)	0.87 (4)	1.15 (7)	0.92 (4)	0.67 (2)	0.30 (3)	26	3.71	1.70
G10	0.33 (6)	0.90 (1)	1.01 (1)	1.33 (5)	1.44 (1)	0.67 (2)	0.20 (4)	20	2.86	2.12
GM	0.38	0.64	0.72	1.25	0.88	0.61	1.65	34.20	4.89	2.59
10% PEG Treatment										
Genotype	**GE (%)**	**GC (%)**	**SH (cm)**	**RL (cm)**	**NR**	**FSW (g)**	**DSW (g)**	**RkSum**	**RkM**	**RkSD**
G1	0	0.50 (5)	0.23 (6)	0.37 (6)	0.29 (7)	0.03 (4)	0.003 (4)	32	5.33	1.21
G2	0	0.80 (2)	0.72 (1)	0.67 (5)	0.78 (3)	0.25 (3)	0.002 (5	19	3.17	1.60
G3	0	1.10 (1)	0.53 (2)	1.96 (1)	0.84 (2)	0.67 (1)	0.006 (3)	10	1.67	0.82
G4	0	0.30 (7)	0.18 (7)	0.18 (7)	0.67 (5)	0.25 (3)	0.025 (1)	30	5.00	2.53
G5	0	0.00 (8)	0.00 (8)	0.00 (8)	0.00 (8)	0.00 (5)	0.000 (6)	46	7.67	0.82
G6	0	0.00 (8)	0.00 (8)	0.00 (8)	0.00 (8)	0.00 (5)	0.000 (6)	46	7.67	0.82
G7	0	0.00 (8)	0.00 (8)	0.00 (8)	0.00 (8)	0.00 (5)	0.000 (6)	46	7.67	0.82
G8	0	0.50 (4)	0.27 (4)	1.67 (2)	0.48 (6)	0.33 (2)	0.015 (2)	20	3.33	1.63
G9	0	0.40 (6)	0.35 (3)	0.73 (4)	1.11 (1)	0.67 (1)	0.003 (4)	19	3.17	1.94
G10	0	0.60 (3)	0.26 (5)	1.23 (3)	0.69 (4)	0.67 (1)	0.003 (4)	20	3.33	1.37
GM	0	0.42	0.25	0.68	0.49	0.29	0.006	2.88	4.80	13.55

RkM below the GM within a column indicates high tolerance; RkM equal to the GM indicates moderate tolerance, while RkM above the GM indicates high susceptibility to drought stress. GM: Grand mean; RkSum: Rank sum; RkM: Rank mean; RkSD: Rank standard deviation. GE: Germination energy; GC: Germination capacity; SH: Shoot height; RL: Root length; NR: Number of roots; FSW: Fresh shoot weight; DSW: Dry shoot weight.

3.2 Stress Tolerance Indices (STIs) of Germination and Seedling Traits and Ranking of EtBr-derived Tomato Genotypes Evaluated for Drought Tolerance under Varying PEG-Induced Stress Conditions

The stress tolerance indices (STIs) for germination and seedling traits, along with the ranking of EtBr-derived tomato genotypes assessed for drought tolerance under different PEG treatments, are shown in Table 6. Under 5% PEG treatment, the highest STI values for germination energy were found in genotype G8 (0.75) and G10 (0.90), while the lowest were seen in G5 (0.27) for germination energy and in G5 (0.40) for germination capacity. For shoot height, G10 had the highest STI (1.01), and G6 had the lowest (0.22). Regarding root length, G8 recorded the highest STI (1.01), and G5 the lowest (0.22). In terms of the number of roots, G10 showed the highest STI (1.73), and G6 the lowest (0.47). For fresh shoot weight, G3 had the highest STI (1.00), while G1 had the lowest (0.10). For dry shoot weight, STI ranged from 0.10 in G5 to 0.75 in G4. Genotypes G2, G3, G8, G9, and G10 had rank mean (RkM) values between 2.86 and 4.29, below the grand mean (GM) of 4.89, indicating high drought tolerance. Conversely, G1, G4, G5, G6, and G7 had RkM values between 5.00 and 6.86, above the GM, suggesting high susceptibility to drought stress at the 5% level.

The 10% PEG treatment results showed that G2 had the highest STI for germination capacity (0.80), while G4 had the lowest (0.30). For shoot height, G2 also recorded the highest STI (0.72), with G4 again showing the lowest (0.18). In terms of root length, G3 had the highest STI value (1.96), whereas G4 had the lowest (0.18). The STI for the number of roots ranged from 1.11 in G9 to 0.29 in G1. The highest STI for fresh shoot weight was observed in G3, G9, and G10 (0.67), while the lowest was in G1 (0.03). For dry shoot weight, STI values varied from 0.025 in G4 to 0.002 in G2. Genotypes G2, G3, G8, G9, and G10 showed RkM values between 1.67 and 3.33, which are below the GM of 4.80, indicating high drought tolerance. Conversely, G1, G4, G5, G6, and G7 had RkMs between 5.00 and 7.67, above the GM, suggesting higher susceptibility to drought stress at the 10% PEG level.

3.3 Estimates of Genetic Parameters of Germination and Seedling Traits of EtBr-derived Tomato Genotypes Evaluated for Drought Tolerance under Varying Peg-Induced Stress Conditions

The estimates of genetic parameters for germination and seedling traits of EtBr-derived tomato genotypes evaluated for drought tolerance under varying PEG-induced stress levels are presented in Table 7. High genotypic coefficients of variation (GCV), ranging from 21.86% to 80.07%, were recorded for all traits except shoot height, which exhibited a moderate GCV of 16.56%. The phenotypic coefficient of variation (PCV) was high across all traits. Broad-sense heritability was also high for most traits, while shoot height (47.46%), root length (59.03%), and dry shoot weight (35.48%) showed moderate heritability. Genetic advance as a percentage of the mean (GAM) was high for all traits, ranging from 23.50% in shoot height to 139.54% in germination energy.

Table 7. Estimates of genetic parameters for germination and seedling traits of EtBr-derived tomato genotypes evaluated for drought tolerance under varying PEG-induced stress conditions

Trait	GM	VGT	GV	PV	GCV (%)	PCV (%)	H^2B (%)	GA	GAM (%)
GE	6.06	18.17	23.55	32.91	80.07	94.66	71.56	8.46	139.54
GC	18.11	50.81	68.95	93.70	45.85	53.45	73.59	14.67	81.03
SH	3.08	0.72	0.26	0.55	16.56	24.03	47.46	0.72	23.50
RL	1.41	0.38	0.20	0.34	31.98	41.62	59.03	0.71	50.62
NR	2.22	0.45	0.41	0.61	28.84	35.21	67.09	1.08	48.67
FSW	0.02	0.000024	0.000019	0.00003	21.86	27.49	63.24	0.01	35.81
DSW	0.002	0.000013	0.0000024	0.0000069	78.17	131.23	34.78	0.0019	95.00

GM: Grand mean; VGT: Genotype × treatment variance; GV: Genotypic variance; PV: Phenotypic variance; GCV: Genotypic coefficient of variation; PCV: Phenotypic coefficient of variation; H^2B: Heritability; GA: Genetic advance; GAM: Genetic advance as percent over the mean. GE: Germination energy; GC: Germination capacity; SH: Shoot height; RL: Root length; NR: Number of roots; FSW: Fresh shoot weight; DSW: Dry shoot weight.

3.4 Pearson’s Correlation of Germination and Seedling Traits of EtBr-derived Tomato Genotypes Evaluated for Drought Tolerance under Varying PEG-Induced Stress Conditions

Table 8 presents the Pearson’s correlation coefficients for germination and seedling traits among EtBr-derived tomato genotypes evaluated for drought tolerance under varying PEG concentrations. Under 0% and 5% PEG (representing no or mild drought stress), significant and strong correlations were observed between germination energy and germination capacity (r = 0.88 and r = 0.82, respectively), as well as between germination capacity and dry shoot weight (r = 0.67). However, under severe drought stress (10% PEG), germination capacity showed strong positive correlations with root length (r = 0.83), shoot height (r = 0.73), number of roots (r = 0.75), fresh shoot weight (r = 0.90), and dry shoot weight (r = 0.80). Additionally, shoot height, root length, and shoot weight were all strongly interrelated under high stress, with correlations such as shoot height to fresh shoot weight (r = 0.81) and root length to dry shoot weight (r = 0.67). This indicates that drought stress intensifies the relationships among germination and growth traits in tomato genotypes.

In this study, ANOVA results revealed significant genotype effects on all evaluated germination and seedling traits, highlighting substantial genetic variability among the tomato genotypes. This variability is essential for future breeding programs aimed at enhancing drought tolerance in tomato (Rasheed et al., 2023). Furthermore, the study demonstrated that higher PEG concentrations led to a marked decline in the performance of germination and seedling traits. These findings are consistent with those of Saima et al. (2018), who reported that germination percentage, shoot weight, dry matter stress index, and plant height stress index all decreased across genotypes with increasing levels of PEG-induced drought stress (5% and 10% PEG).

Table 8. Pearson’s correlation of germination and seedling traits of EtBr-derived tomato genotypes evaluated for drought tolerance under varying PEG-induced stress conditions

0% PEG Treatment							
Traits	**GE (%)**	**GC (%)**	**SH (cm)**	**RL (cm)**	**NR**	**FSW (g)**	**DSW (g)**
GE	1	0.88**	0.53	0.34	0.19	0.17	0.23
GC		1	0.50	0.39	0.29	0.15	0.67*
SH			1	0.49	0.29	0.35	0.37
RL				1	0.25	0.49	0.33
NR					1	0.27	-0.16
FSW						1	0.27
DSW							1
5% PEG Treatment							
Trait	**GE (%)**	**GC (%)**	**SH (cm)**	**RL (cm)**	**NR**	**FSW (g)**	**DSW (g)**
GE	1	0.82**	0.31	0.35	0.30	-0.29	-0.36
GC		1	0.55	0.37	0.40	-0.14	-0.40
SH			1	0.30	0.53	0.06	-0.34
RL				1	-0.06	0.08	-0.42
NR					1	-0.03	-0.16
FSW						1	0.29
DSW							1
10% PEG Treatment							
Trait	**GC (%)**	**SH (cm)**	**RL (cm)**	**NR**	**FSW (g)**	**DSW (g)**	
GC	1	0.73*	0.83**	0.75*	0.90**	0.80**	
SH		1	0.63*	0.66*	0.81**	0.73*	
RL			1	0.40	0.70*	0.67*	
NR				1	0.84**	0.57	
FSW					1	0.78**	
DSW						1	

**: Significant at P ≤ 0.05; **: Significant at P ≤ 0.01. GE: Germination energy; GC: Germination capacity; SH: Shoot height; RL: Root length; NR: Number of roots; FSW: Fresh shoot weight; DSW: Dry shoot weight.*

As drought stress increased through higher PEG concentrations, all tomato genotypes exhibited a clear and progressive decline in germination and seedling traits. Grand means for germination energy dropped from 11.67% at control (0% PEG) to 0% at 10% PEG, with similar reductions observed in germination capacity, shoot and root growth, and biomass. Under control conditions, genotypes G7 and G9 demonstrated the highest vigour, showing the greatest germination capacity and energy, while G5 also performed notably well for shoot height. In contrast, G6 consistently showed poor performance even in the absence of stress, underscoring substantial genotypic differences in drought tolerance potential.

This pattern of decline continued under moderate drought stress (5% PEG), where G7 and G9 maintained relatively high germination capacity and energy, as well as superior shoot and root growth, indicating moderate resilience to drought. Conversely, G6 exhibited a complete loss of germination energy and very low values for other traits, reflecting high sensitivity even to moderate stress. When exposed to severe drought (10% PEG), most genotypes failed to germinate or grow. However, G3 and G9 stood out with the highest germination capacity; G3 achieved the longest roots, while G9 produced the most roots. A few other genotypes (G1, G2, G4, G8, and G10) showed minimal but measurable growth, with G8 and G10 maintaining some root and shoot development. Overall, although germination and growth traits declined sharply with increasing stress, G3 and G9 consistently retained higher values, highlighting their potential as valuable sources of drought tolerance. The observed reduction in trait performance is likely due to PEG-induced water unavailability, which is essential for germination. This water deficit leads to decreased hydrolysis of seed reserve materials and, consequently, lower germination percentages (Eesha et al., 2024). Notably, tolerant genotypes with high germination energy and capacity demonstrated more rapid and robust germination compared to sensitive genotypes, a finding consistent with previous results in tomato (Kumar et al., 2017).

Root length, in particular, emerged as a critical trait for drought tolerance. Extensive root growth is indicative of enhanced drought adaptation, as early and rapid root elongation enables plants to access deeper soil moisture under water-limited conditions (Kumar et al., 2017; Kim et al., 2001). In this study, certain genotypes—such as G3 and G9—exhibited long and well-developed roots under both 5% and 10% PEG treatments, likely reflecting their inherent tolerance mechanisms. These observations align with the findings of Kulkarni and Deshpande (2008), who reported similar results in tomato.

Stress tolerance indices (STIs) are effective tools for identifying genotypes that perform well under both stress and non-stress conditions (Fernandez, 1992), offering valuable insights for breeders targeting resilience in challenging environments. In this study, STIs were calculated using germination and seedling traits, and genotypes were ranked based on mean and ranked mean values under 5% and 10% PEG-induced drought stress. Genotypes G8, G9, and G10 showed strong tolerance at 5% PEG, while G3, G2, G9, G8, and G10 performed best at 10% PEG. G10 excelled under moderate stress, and G3 under severe stress, particularly in germination capacity and root length. In contrast, G5, G6, and G7 were drought-sensitive. Root length and germination capacity emerged as reliable indicators of drought tolerance, consistent with findings by Ferioun et al. (2024) in barley and Moradi et al. (2012) in maize.

These results suggest that tomato genotypes G3, G8, G9, and G10, which showed strong drought tolerance across different stress levels, should be prioritized as parental lines in breeding programs. Emphasizing traits like root length and germination capacity during selection can improve the efficiency of identifying drought-tolerant plants, while screening under varying drought intensities helps select lines with stable and robust tolerance. Conversely, susceptible genotypes such as G5, G6, and G7 should be excluded from breeding pipelines to concentrate resources on superior candidates.

Importantly, the drought-tolerant genotypes identified—G3, G8, G9, and G10—were all derived from ethidium bromide (EtBr)-induced mutagenesis. These genotypes originated from two parental lines—NG/AA/SEP/09/042 (G1–G5) and AKUNGBA2 (G6–G10)—subjected to varying durations (0–96 hours) of 0.5% EtBr treatment. The most resilient genotypes were mutants exposed to intermediate or prolonged EtBr durations (48–96 hours), suggesting that mutagenesis may have introduced beneficial genetic variations enhancing key traits like germination capacity and root length. In contrast, untreated parental lines (G1 and G6) and those with minimal exposure showed lower drought tolerance. This highlights the potential of EtBr-induced mutagenesis, when integrated with conventional breeding and phenotypic screening, to accelerate the development of drought-resilient tomato varieties. Selecting mutants from optimized EtBr exposure durations could thus enhance the efficiency of breeding programs targeting stress adaptation.

Estimating genetic parameters under drought stress is crucial for breeding drought-tolerant tomato varieties. By identifying traits mainly controlled by genetic factors rather than environmental influences, breeders can concentrate on characteristics that are more consistently inherited and more responsive to selection. The difference between phenotypic (PCV) and genotypic (GCV) variation helps measure trait variability, but heritability combined with genetic advance as a percentage of the mean (GAM) offers a clearer understanding of genetic control and the potential for improvement (Ajayi et al., 2017).

In this study, key traits such as germination energy, germination capacity, root length, number of roots, fresh shoot weight, and dry shoot weight showed substantial genetic variability and high heritability (>60%), indicating strong genetic influence and stable inheritance under drought conditions. The high genetic advance observed for these traits suggests that additive gene effects predominate, making them excellent targets for selection in breeding programs. This means that selecting for these traits is likely to result in significant genetic gains and improved drought tolerance in subsequent generations. These findings align with previous studies (e.g., Bayoumi et al., 2008; Rajarajan et al., 2018), reinforcing the value of focusing on traits with high heritability and genetic advance for effective drought tolerance breeding. Overall, the results emphasize that breeding strategies should prioritize traits governed by additive genetic effects and exhibit substantial genetic variability to develop tomato genotypes with enhanced resilience to drought stress.

Correlation analysis is a valuable tool in plant breeding because it reveals key trait associations that enable the simultaneous improvement of multiple traits through indirect selection (Zhang et al., 2021). In this study, conducted under severe drought stress, germination capacity proved to be a reliable predictor of overall seedling vigour and biomass, specifically affecting shoot height, root length, number of roots, fresh shoot weight, and dry shoot weight. These results suggest that selecting for germination capacity under stress conditions can significantly improve drought tolerance in tomato genotypes. Interestingly, correlation values were generally stronger and more significant under severe drought stress than under control conditions, indicating that stress enhances trait interdependence. This contrasts with findings in maize reported by Badr et al. (2020), where trait correlations weakened under stress. In our study, the close relationship between germination and subsequent seedling growth traits emphasizes the importance of including both germination and seedling vigour traits in selection strategies, especially under different levels of stress.

Furthermore, since these results were obtained from mutants induced by ethidium bromide (EtBr), it is important to use multi-trait screening methods to account for potentially changed genetic relationships. Mutation breeding, as shown by Ajie (2025), can break undesirable linkages and create new trait combinations by disrupting existing trait associations. This approach is especially useful in reducing negative correlations that may hinder the simultaneous improvement of yield-related traits.

The importance of germination traits in affecting seedling performance under drought stress has also been highlighted in previous studies on wheat (Khan et al., 2013) and rice (Bernad et al., 2023), further supporting the idea that germination capacity is key to drought resilience. Therefore, breeding programs should focus on enhancing germination capacity, especially under high-stress conditions, while also accounting for complementary seedling traits under milder stress. Such an integrated approach to trait selection will improve the development of drought-tolerant tomato varieties.

4. CONCLUSION

This study showed that ethidium bromide (EtBr)-induced mutagenesis effectively created significant genetic diversity for drought tolerance among tomato genotypes. Important traits like germination capacity, root length, and shoot weight showed high heritability and genetic gain, indicating strong additive genetic control and making them proper for selection.

Under drought stress simulated by PEG, genotypes G3, G8, G9, and G10 were consistently performed better than others, especially in germination and root traits, making them promising for drought-tolerant breeding. Correlation analysis further confirmed germination capacity as a reliable predictor of seedling vigour and biomass under stress, emphasizing its role in selection strategies.

Breeding programs focused on improving drought tolerance in tomato should combine chemical mutagenesis with early screening for germination and seedling vigour traits. Genotypes G3, G8, G9, and G10 should be prioritized as parent lines, with germination capacity and root length serving as key selection criteria. Future efforts should also include multi-trait and stress-level screening to develop high-performing, drought-resistant tomato varieties suitable for cultivation in Nigeria's drought-prone areas.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

The author (s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

COMPETING INTERESTS

Authors have declared that they have no known competing financial interests or non-financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Peer-review history:
The peer review history for this paper can be accessed here:
https://pr.sdiarticle5.com/review-history/142764