Research Square

Preprints are preliminary reports that have not undergone peer review.
They should not be considered conclusive, used to inform clinical practice,
or referenced by the media as validated information.

ISSR-Based Genetic Characterization and Physiological Evaluation of PEG-Selected Tomato Regenerants under Deficit Irrigation

Ola Alnaddaf

ola.naddaf@damascusuniversity.edu.sy

Damascus University

Hisham Al-Atwani

General Commission for Scientific Agricultural Research

Wasim Mohsen

General Commission for Scientific Agricultural Research

Research Article

Keywords: Solanum lycopersicum, in vitro selection, polyethylene glycol, somaclonal variation, ISSR markers, deficit irrigation

Posted Date: November 18th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-11414259/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License.
Read Full License

Additional Declarations: No competing interests reported.

ISSR-Based Genetic Characterization and Physiological Evaluation of PEG-Selected Tomato Regenerants under Deficit Irrigation

Ola Alnaddaf*[1], Hisham Al-Atwani[2], Wasim Mohsen[2]

[1]The Second Faculty of Agriculture, Damascus University, As-Suwayda, Syria, E-mail: ola.naddaf@damascusuniversity.edu.sy

[2] Researcher, General Commission for Scientific Agricultural Research, As-Swayda, Syria

Corresponding author: Ola Alnaddaf; The Second Faculty of Agriculture, Damascus University, As-Suwayda – Syria. E-mail: ola.naddaf@damascusuniversity.edu.sy

ORCID**:** 0000-0003-3854-8138

Abstract

Background: Water deficit is a major constraint on tomato (*Solanum lycopersicum* L.) production. *In vitro* selection using polyethylene glycol (PEG) may identify regenerated lines with favorable responses to limited water availability. This study combined field evaluation and inter-simple sequence repeat (ISSR) analysis to characterize phenotypic performance and molecular variation in PEG-selected tomato regenerants and their donor cultivars.

Methods: Four regenerated lines (DH1, BR1, DA1, DA2), derived from three tomato cultivars following *in vitro* selection under PEG-induced osmotic stress, were evaluated alongside their donor cultivars. The seven genotypes were grown under full irrigation (100% field capacity) and deficit irrigation (75% field capacity). Morphological and physiological traits—plant height, stem diameter, branch number, leaf area, dry weight, chlorophyll content, relative water content (RWC), and proline concentration—were measured. Genetic variation was assessed using nine informative ISSR primers;

amplification profiles were used to estimate polymorphism, polymorphism information content (PIC), and genetic similarity.

Results: Deficit irrigation affected all measured traits, with responses varying among genotypes. DH1 exhibited a smaller reduction in RWC than its donor cultivar, whereas DA2 showed comparatively small reductions in plant height and leaf area. Proline concentration increased under deficit irrigation in all genotypes. ISSR analysis generated 39 bands, of which 27 were polymorphic (69.23% polymorphism). Mean PIC across primers was 0.248, with the highest values (0.408) for ISSR812 and ISSR866. Unique bands were detected in the donor cultivar Daraa and regenerated line DA2. Nei and Li similarity coefficients between donors and regenerants ranged from 0.541 to 0.703, indicating varying genetic divergence.

Conclusion: The regenerants exhibited variation in phenotypic responses to deficit irrigation and ISSR profiles. DH1 and DA2 displayed comparatively favorable responses in selected traits, supporting further evaluation under water-limited conditions. However, the results do not establish that PEG selection alone caused the observed variation or demonstrate improved yield or overall drought tolerance. Multi-season and multi-location trials incorporating reproductive performance, fruit yield, fruit quality, and water-use efficiency are needed to determine agronomic value. Nevertheless, these regenerated lines represent promising genetic resources for tomato breeding programs targeting water-limited environments.

Keywords: *Solanum lycopersicum*; *in vitro* selection; polyethylene glycol; somaclonal variation; ISSR markers; deficit irrigation.

1.Introduction

Water deficit is among the most severe abiotic stresses limiting crop growth, development, and productivity worldwide [25, 1]. Tomato (*Solanum lycopersicum* L.) is particularly sensitive to water deficit, which can adversely affect vegetative growth, physiological processes, yield, and fruit quality [4, 5]. Conventional breeding for drought tolerance remains challenging because plant responses to water deficit are complex quantitative traits controlled by multiple genes and strongly influenced by environmental conditions and genotype × environment interactions [2, 35].

Biotechnological approaches can complement conventional breeding by providing opportunities to generate and select novel genetic variation associated with stress adaptation [3]. Plant tissue culture, particularly regeneration through a callus phase, can generate somaclonal variation that represents a potential source of genetic diversity for crop improvement [31, 12]. The frequency and nature of somaclonal variation may be affected by genotype, explant source, duration of culture, plant growth regulator composition, and selection pressure [40, 26]. Such variation may arise through chromosomal rearrangements, DNA sequence mutations, transposable element activity, and epigenetic changes occurring during tissue culture [9].

Phenotypic evaluation of regenerated plants is an important first step in identifying potentially useful variants. However, morphological and physiological differences alone cannot reliably determine whether observed variation reflects stable genetic changes or phenotypic responses to environmental conditions [23]. Molecular characterization is therefore important for detecting genetic variation among regenerated plants and determining their genetic relationships with the corresponding donor genotypes [6].

DNA-based molecular markers provide useful tools for detecting genetic variation at the genome level. Among the available marker systems, inter-simple sequence repeat (ISSR) markers are

particularly useful for assessing genetic variation because they generally provide high levels of polymorphism and reproducibility, while being technically simple and not requiring prior knowledge of genome sequence information [14,33]. ISSR markers have been successfully used to assess genetic fidelity and detect somaclonal variation in several crop species, including tomato and other Solanum species [18].

In previous study [4], *in vitro* selection under polyethylene glycol (PEG 6000)-induced osmotic stress was used to select callus lines selected under PEG-induced osmotic stress from tomato genotypes. Four regenerated lines, namely DH1, BR1, DA1, and DA2, were subsequently obtained from PEG-selected callus material. Although these lines were selected under controlled *in vitro* stress conditions, their genetic divergence from the corresponding donor plants and their performance under field water-deficit conditions had not yet been established.

The present study aimed to integrate molecular characterization with field-based evaluation of PEG-selected tomato regenerants under full and deficit irrigation. Specifically, the objectives were to: (i) evaluate morphological and physiological responses of donor cultivars and regenerated lines under both irrigation regimes; (ii) characterize ISSR polymorphism and genetic relationships among the genotypes to assess somaclonal variation; and (iii) identify regenerants warranting further evaluation for drought-tolerance breeding.

2. Materials and Methods

2.1. Plant Material and Origin of PEG-Selected Regenerated Lines

The study was conducted at the tissue culture laboratories of the National Commission for Biotechnology (NCBT) and the General Commission for Scientific Agricultural Research (GCSAR), Syria. Three tomato (*Solanum lycopersicum* L.) cultivars, Daher-Aljabal (DH), Brieh (BR), and Daraa (DA), and four PEG-selected regenerated lines (DH1, BR1, DA1, DA2) were evaluated.

The regenerated lines were previously obtained through *in vitro* selection of callus under variety-specific sub-lethal concentrations of PEG 6000, as described in detail by Alnaddaf et al. [4]. Briefly, callus cultures were induced from root explants of 15-day-old seedlings on Murashige and Skoog (MS) medium supplemented with 2 mg/L BAP and 0.2 mg/L NAA. Two-month-old homogeneous callus cultures were subjected to PEG-induced osmotic stress at concentrations of 2%, 4%, 6%, and 8% for eight weeks. Sub-lethal PEG concentrations (4%, 6%, and 8%) were determined based on approximately 50% reduction in callus relative growth rate and cell viability. DH1 originated from Daher-Aljabal callus selected at 4% PEG, BR1 from Brieh callus selected at 6% PEG, and DA1 and DA2 from Daraa callus selected at 6% and 8% PEG, respectively (Table 1). Regeneration was achieved on MS medium supplemented with zeatin and gibberellic acid (GA_3).

Table 1. Plant material and origin of regenerated lines

Donor cultivar	Regenerated line	PEG concentration used for selection
Daher-Aljabal (DH)	DH1	4%
Brieh (BR)	BR1	6%
Daraa (DA)	DA1	6%
Daraa (DA)	DA2	8%

2.2. Acclimatization stage

Regenerated tomato plantlets were acclimatized following the procedure described previously , with minor modifications where necessary. Briefly, regenerated shoots were transferred to rooting medium consisting of Murashige and Skoog (MS) medium supplemented with 1 mg L^{-1} indole-3-butyric acid (IBA) and maintained for 60 days to promote root development. Rooted plantlets were carefully removed from the culture vessels, and residual agar was washed off with distilled water. The plantlets were then transferred to pots containing a sterilized peat moss–perlite mixture (1:1, v/v) and maintained under greenhouse conditions. Relative humidity was initially maintained at a high level using transparent polyethylene covers and gradually reduced to facilitate acclimatization to *ex*

vitro conditions. The acclimatized plants were subsequently grown under greenhouse conditions before being transplanted to the field for evaluation under the two irrigation regimes.

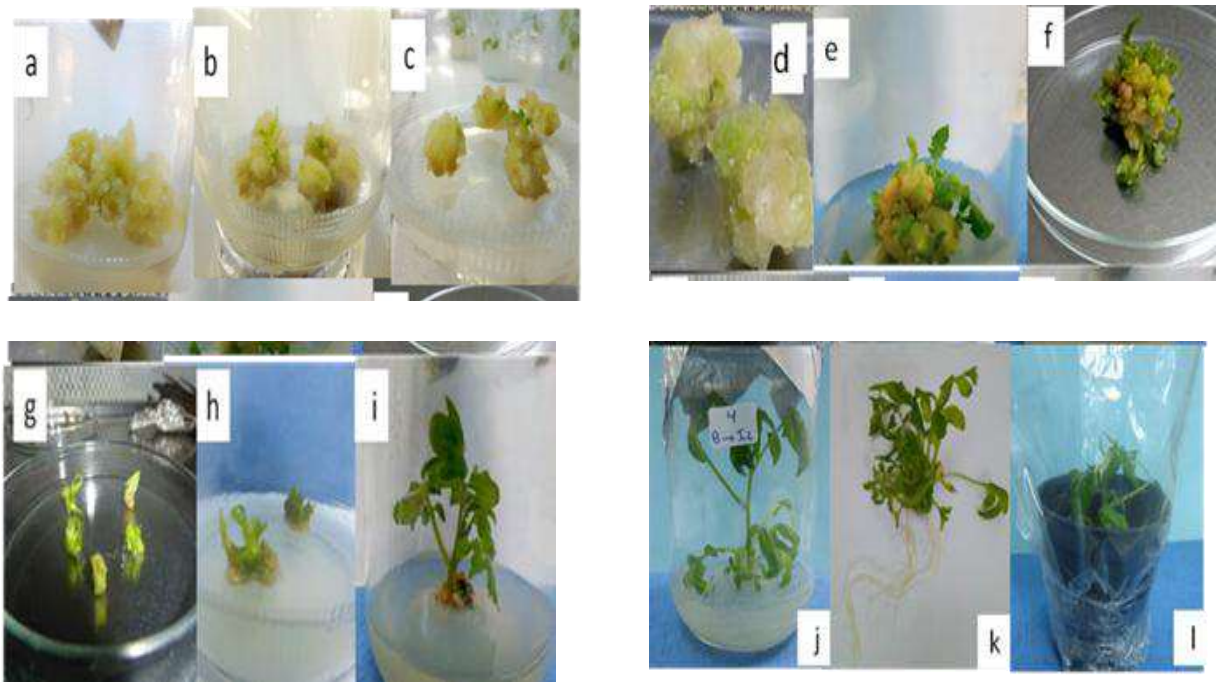


Figure 1. Stages of *in vitro* selection and plant regeneration (reproduced from Alnaddaf et al.). (a–d) Callus formation and proliferation from root explants; (e, f) shoot formation; (g) regenerated shoots cut aseptically to remove callus; (h) culture on multiplication medium; (i) growth of regenerated shoots; (j) rooting of regenerated shoot; (k) rooted plantlet removed from medium and washed to remove agar; (l) acclimatization of rooted plantlet.

2.3. Field Experiment

2.3.1. Experimental Design and Field Conditions

Acclimatized plantlets approximately 15–20 cm in height were transplanted to a field with 40 cm spacing between plants and rows. The experiment was conducted in clay-loam volcanic soil, and soil physicochemical properties at depths of 0–30 and 30–60 cm are presented in Table 2.

The experiment was arranged in a randomized complete block design (RCBD) with three replicates (block) and 10 plants per replicate.

Table 2. Physicochemical properties of the experimental soil.

Soil property	0–30 cm	30–60 cm
Organic matter (%)	1.422	1.094
Phosphorus (ppm)	53.5	33.5
Potassium (ppm)	270	230
Soil pH	7.62	7.56
Sand (%)	30	30
Silt (%)	18	12
Clay (%)	52	58

2.3.2. Irrigation Treatments

Two irrigation regimes were applied: full irrigation (100% field capacity) and deficit irrigation (75% field capacity). Field capacity was determined as 39.4% (v/v). Soil moisture was monitored using tensiometers, and irrigation was applied when soil water depletion reached approximately 80% of available water capacity.

Drip irrigation was supplied through 16-mm-diameter pipelines with emitters spaced 40 cm apart and a discharge rate of 3.5 L h^{-1}. Seasonal irrigation amounts were 327.9 mm under full irrigation and 245.9 mm under deficit irrigation, representing an approximately 25% reduction in water supply.

2.3.3. Morphological and Physiological Measurements

Morphological and physiological traits were evaluated after two months of field growth. The measured morphological traits included plant height (cm), stem diameter (mm), number of lateral shoots (shoots $plant^{-1}$), leaf area (cm^2), and plant dry weight (g). Leaf area was measured using a LI-COR 3100 Area Meter (LI-COR, Lincoln, NE, USA). Plant dry weight was determined by drying the sampled plant material at 105 °C until constant weight.

Total chlorophyll concentration was determined spectrophotometrically according to Lichtenthaler [24] using 80% acetone, and chlorophyll concentration was calculated using the corresponding equations. Relative water content (RWC) was determined according to Blum [11] using fresh, turgid, and dry leaf weights. Free proline concentration was determined using the colorimetric method of

Bates et al. [8], with absorbance measured at 520 nm. For each replicate, measurements were taken from five randomly selected plants, and the mean value was used for statistical analysis.

2.3.4. Statistical Analysis

To quantify the relative response of each genotype to deficit irrigation, reduction rates were calculated for traits that decreased under water deficit (plant height, stem diameter, number of branches, leaf area, dry weight, chlorophyll content, and relative water content) as:

$$\text{Reduction Rate (\%)} = [(\text{Value at 100\% FC} - \text{Value at 75\% FC}) / \text{Value at 100\% FC}] \times 100$$

For proline concentration, which increased under deficit irrigation, the increase rate was calculated:

$$\text{Increase Rate (\%)} = [(\text{Value at 75\% FC} - \text{Value at 100\% FC}) / \text{Value at 100\% FC}] \times 100$$

Reduction and increase rates were calculated for each replicate and then subjected to analysis of variance (ANOVA) using GenStat software Version12 and differences among mean values were assessed using the LSD test, with significance set at $p \leq 0.05$.

2.4. Genetic Variation Analysis Using ISSR Markers

2.4.1. DNA Extraction and Quality Assessment

Genomic DNA was extracted from bulked leaf samples of five randomly selected plants per genotype. Approximately 0.2 g of young fresh leaf tissue was ground in liquid nitrogen and incubated in extraction buffer containing 100 mM Tris-HCl, 1.4 M NaCl, 20 mM EDTA, 2% CTAB, and 0.2% β-mercaptoethanol at 65°C for 60 min. DNA was purified using chloroform alcohol (24:1), precipitated with cold isopropanol, washed with 70% ethanol, dissolved in TE buffer, and treated with RNase. DNA was stored at −20°C until analysis.

DNA concentration and purity were assessed spectrophotometrically at 260 and 280 nm, and DNA integrity was checked by electrophoresis on 1% agarose gel in 1× TBE buffer. DNA concentration was calculated as:

$$\text{DNA } (\mu g\ \mu L^{-1}) = (A260 \times 50 \times \text{dilution factor}) / 1000.$$

2.4.2. ISSR-PCR Amplification

Twelve ISSR primers were tested for amplification (Table 3). Each 25-μL PCR reaction contained 50 ng genomic DNA, 2.5 μL 10× PCR buffer, 2 μL $MgCl_2$ (25 mM), 2 μL dNTPs (10 mM μL^{-1}), 1 μL primer (5 μM), and 0.3 μL Taq DNA polymerase (5 U μL^{-1}). PCR amplification was performed using an Eppendorf Mastercycler Nexus Gradient PCR system.

The amplification program consisted of an initial denaturation at 94°C for 5 min, followed by 45 cycles of 94°C for 1 min, 50–55°C for 1 min, and 72°C for 1.5 min, with a final extension at 72°C for 10 min, according to Erdinc et al. [16].

Table 3. ISSR primers used in the study.

Primer	Sequence	Primer	Sequence
ISSR866	$(CTC)_6$	ISSR A830241	$(ACTG)_5$
ISSR8082	$(CT)_9G$	ISSR830	$(TG)_8G$
ISSR nlssr3	$(CAG)_5$	ISSR811	$(GA)_8C$
ISSR862	$(AGC)_6$	ISSR812	$(GA)_8A$
ISSR10	$(AC)_8G$	ISSR813	$(CT)_8C$
ISSR23	$GAG(TC)_8$	ISSR W814	$(CT)_8TC$

2.4.3. Electrophoresis and Band Scoring

PCR products were separated on 1.5% agarose gels in 1× TBE buffer and visualized under UV light following ethidium bromide staining. A 100-bp DNA ladder was used as a molecular-size marker. Clear and reproducible bands were scored as present (1) or absent (0) to generate a binary matrix.

2.4.4. Molecular Data Analysis

The number of amplified and polymorphic bands and percentage polymorphism were determined for each primer using XLSTAT. GelAnalyzer software was used for band scoring. Genetic similarity among donor plants and regenerated lines was estimated using the Nei and Li [30] coefficient, and cluster analysis was performed using the Unweighted Pair Group Method with Arithmetic Mean (UPGMA).

Polymorphism information content (PIC) was calculated according to Anderson et al. (1993):

$$PIC = 1 - \frac{\sum_{i=1}^{n} p_i^2 + \sum_{i=1}^{n} q_i^2}{n}$$

where $p_i$and $q_i$represent the frequencies of the presence and absence, respectively, of the i-th ISSR band, and n represents the total number of scored bands.

3. Results

3.1. Field Evaluation of PEG-Selected Regenerated Lines and Donor Cultivars

The four regenerant lines (DH1, BR1, DA1, and DA2) obtained under PEG-selection [4] were evaluated under field conditions alongside their respective donor cultivars.

3.1.1. Morphological and Physiological Performance under Full Irrigation (100% Field Capacity)

Significant differences were observed among donor cultivars and PEG-selected regenerated lines for most morphological and physiological traits evaluated under full irrigation conditions (Table 4). In general, regenerated lines exhibited lower vegetative growth than their corresponding donor cultivars, although the magnitude of the response varied among genotypes. However, several traits, including branch number, leaf area, and chlorophyll content in the Daher Aljabal-derived genotypes, did not differ significantly between regenerated lines and their donors.

Daher Aljabal genotype: The regenerated line DH1 exhibited significantly lower plant height (41.80 vs. 52.27 cm), stem diameter (4.22 vs. 5.38 mm), and relative water content (61.50 vs. 66.40%) than its donor cultivar DH. Dry weight (38.7 vs. 32.7 g) and proline concentration (127.84 vs. 112.31 µmol/g FW) were numerically higher in DH1, although these differences were not statistically significant. Branch number, leaf area, and chlorophyll content did not differ significantly between the two genotypes.

Brieh genotype: The regenerated line BR1 showed significantly lower plant height (81.50 vs. 91.27 cm), stem diameter (4.81 vs. 6.10 mm), branch number (11.67 vs. 16.00), leaf area (62.83 vs. 66.40 cm²), and chlorophyll content (2.27 vs. 3.17 mg/g FW) than its donor cultivar BR. Relative water content was also

lower in BR1 (71.60 vs. 73.60%), whereas proline concentration was significantly higher (99.32 vs. 79.66 µmol/g FW).

Daraa genotype**:** Both regenerated lines (DA1 and DA2) exhibited significantly lower plant height than the donor cultivar. DA2 showed a significant reduction in stem diameter (4.66 mm), whereas DA1 (5.21 mm) remained statistically comparable to the donor cultivar (5.87 mm). No significant differences were detected in leaf area among DA, DA1, and DA2. The regenerated line DA1 recorded the highest chlorophyll content (3.75 mg/g FW) and relative water content (78.57%) among the Daraa-derived genotypes. In contrast, DA2 exhibited the highest proline concentration (55.27 µmol/g FW) compared with DA1 (35.58 µmol/g FW) and the donor cultivar (28.66 µmol/g FW).

Comparison among regenerated lines: Considerable variation in field performance was observed among the four regenerated lines. BR1 exhibited the greatest plant height (81.50 cm), whereas DA1 tended to show higher values for stem diameter (5.21 mm), branch number (12.67), chlorophyll content (3.75 mg/g FW), and relative water content (78.57%). The highest dry weight was recorded in BR1 (59.3 g), while DH1 accumulated the highest proline concentration (127.84 µmol/g FW).

Table 4. Morphological and physiological traits of donor cultivars and PEG-selected regenerated tomato lines under full irrigation (100% field capacity).

Genotype	Plant height cm	Stem diameter mm	Number of branches	Leaf area (cm^2)	Dry weight g	Chlorophyll content (mg/g FW)	RWC (%)	Proline content µmol/g FW
DH	52.27e	5.38ab	11.67bc	53.60d	32.7d	2.26c	66.40d	112.31a
DH1	41.80g	4.22c	8.33c	51.50d	38.7d	1.97c	61.50e	127.84a
BR	91.27a	6.10a	16.00a	66.40b	69.0a	3.17ab	73.60bc	79.66b
BR1	81.50b	4.81bc	11.67b	62.83c	59.3ab	2.27c	71.60c	99.32b
DA	64.53c	5.87a	14.00ab	76.67a	43.0cd	3.61ab	76.67ab	28.66c
DA1	58.83d	5.21ab	12.67b	75.43a	53.7bc	3.75a	78.57a	35.58c
DA2	48.60f	4.66bc	9.67c	74.47a	32.7d	3.06b	74.47abc	55.27bc
$LSD_{0.05}$	3.077	0.895	2.172	3.436	11.73	0.572	4.111	5.958

Means followed by different letters within a column are significantly different at $P \leq 0.05$.

3.1.2. Morphological and Physiological Responses to Deficit Irrigation (75% Field Capacity)

Deficit irrigation (75% field capacity) significantly affected all morphological and physiological traits evaluated during the vegetative growth stage. However, the magnitude of the response differed among genotypes. In general, PEG-selected regenerated lines exhibited smaller reductions in growth and physiological parameters than their corresponding donor cultivars, indicating improved tolerance to water deficit conditions.

Plant height and stem diameter. Plant height decreased under deficit irrigation in all genotypes (Table 5). The regenerated line DH1 exhibited a significantly smaller reduction (19.14%) than its donor cultivar Daher Aljabal (27.17%). Likewise, DA2 showed the lowest reduction among the Daraa-derived genotypes (8.60%), differing significantly from both DA1 (17.76%) and the donor cultivar (18.92%). In contrast, no significant differences were detected between Brieh (15.73%) and BR1 (14.72%).

Stem diameter was less affected by deficit irrigation than plant height. No significant differences were observed between donor cultivars and regenerated lines derived from Daher Aljabal or Brieh. Among the Daraa-derived genotypes, DA1 exhibited the smallest reduction in stem diameter (2.11%), whereas the donor cultivar Daraa showed the greatest reduction (15.50%).

Table 5. Effect of deficit irrigation on plant height and stem diameter of donor cultivars and PEG-selected regenerated lines.

Genotype	Plant height cm		Reduction Rate (%)	Stem diameter mm		Reduction Rate (%)
	100% FC	75% FC		100% FC	75% FC	
DH	52.27e	38.07f	27.17c	5.38ab	4.92b	8.55ab
DH1	41.80g	33.80g	19.14b	4.22c	4.02d	4.74ab
BR	91.27a	76.91a	15.73b	6.10a	5.61a	8.03ab
BR1	81.50b	69.50b	14.72b	4.81bc	4.66bc	3.12ab
DA	64.53c	52.32c	18.92b	5.87a	4.96b	15.50b
DA1	58.83d	48.38d	17.76b	5.21ab	5.10b	2.11a
DA2	48.60f	44.42e	8.60a	4.66bc	4.32cd	7.29ab
$LSD_{0.05}$	3.077	2.703	5.518	0.895	0.417	12.08

Reduction rate (%) = [(value at 100% FC − value at 75% FC) / value at 100% FC] × 100. Means followed by different letters within a column are significantly different at $P \leq 0.05$.

Number of branches and leaf area. Deficit irrigation reduced branch number in all genotypes (Table 6). The regenerated line DH1 showed a significantly smaller reduction (2.0%) than its donor cultivar (9.59%). However, no significant differences were detected between Brieh and BR1 or among Daraa, DA1, and DA2.

Leaf area was among the most affected traits under deficit irrigation. Regenerated lines generally exhibited smaller reductions in leaf area than their corresponding donor cultivars. The lowest reduction was recorded in DA2 (6.91%), followed by DA1 (11.84%) and BR1 (16.73%). In contrast, the donor cultivars exhibited greater reductions, particularly Daher Aljabal (41.04%), followed by Brieh (28.61%) and Daraa (22.17%). Significant differences were observed among genotypes with respect to leaf area reduction under water deficit.

Table 6. Effect of deficit irrigation on number of branches and leaf area of donor cultivars and PEG-selected regenerated lines.

Genotype	Number of branches		Reduction Rate (%)	Leaf area (cm^{2})		Reduction Rate (%)
	100% FC	75% FC		100% FC	75% FC	
DH	11.67bc	10.55b	9.59b	53.60d	31.60h	41.04d
DH1	8.50c	8.33c	2.00a	51.50d	38.09j	26.04c
BR	16.00a	14.36a	10.25b	66.40b	47.40f	28.61c
BR1	11.67b	10.64b	8.83b	62.83c	52.32e	16.73b
DA	14.00ab	13.50a	3.57a	76.67a	59.67c	22.17c
DA1	13.20b	12.67ab	4.02ab	75.43a	66.50b	11.84b
DA2	9.67c	9.20bc	4.86ab	74.47a	69.32a	6.91a
$LSD_{0.05}$	2.172	1.766	3.74	3.436	2.056	3.622

Reduction rate (%) = [(value at 100% FC − value at 75% FC) / value at 100% FC] × 100. Means followed by different letters within a column are significantly different at $P \leq 0.05$.

Dry weight and chlorophyll content. Dry weight decreased under deficit irrigation in all genotypes (Table 7). The regenerated line BR1 exhibited a significantly smaller reduction (1.59%) than its donor cultivar Brieh (5.06%). Similar trends were observed for DH1 (3.73%) relative to Daher Aljabal (4.59%) and for DA1 (2.14%) and DA2 (2.09%) relative to Daraa (2.79%), although these differences were not statistically significant.

Chlorophyll content also declined under deficit irrigation. The greatest reduction was observed in Brieh (24.61%), whereas BR1 exhibited a significantly smaller decrease (9.25%). Among the Daraa-derived genotypes, chlorophyll reductions ranged from 4.07% to 7.73% and did not differ significantly. Likewise, no significant differences were detected between Daher Aljabal (12.39%) and DH1 (11.17%).

Table 7. Effect of deficit irrigation on dry weight and chlorophyll content of donor cultivars and PEG-selected regenerated lines.

Genotype	Dry weight (g)		Reduction Rate (%)	chlorophyll content (mg/g FW)		Reduction Rate (%)
	100% FC	75% FC		100% FC	75% FC	
DH	32.70d	31.19j	4.59b	2.26c	1.98e	12.39a
DH1	38.70d	37.26e	3.73ab	1.97c	1.75f	11.17a
BR	69.00a	65.51a	5.06b	3.17ab	2.39d	24.61b
BR1	59.3ab	58.36b	1.59a	2.27c	2.06e	9.25a
DA	43.00cd	41.80d	2.79ab	3.61ab	3.36b	6.92a
DA1	53.70bc	52.55c	2.14ab	3.75a	3.46a	7.73a
DA2	32.70d	32.02f	2.09ab	3.19b	3.06c	4.07a
$LSD_{0.05}$	11.73	1.677	3.936	0. 572	0.089	10.39

Reduction rate (%) = [(value at 100% FC − value at 75% FC) / value at 100% FC] × 100. Means followed by different letters within a column are significantly different at $P \leq 0.05$.

Relative water content and proline accumulation. Relative water content (RWC) decreased in all genotypes under deficit irrigation (Table 8). The regenerated line DH1 exhibited a significantly smaller reduction (3.17%) than its donor cultivar Daher Aljabal (18.19%). Similar trends were observed for BR1 and DA2; however, these differences were not statistically significant.

Proline concentration increased in response to deficit irrigation in all genotypes. Donor cultivars generally exhibited greater relative increases in proline accumulation than regenerated lines. Proline increased by 24.97%, 22.11%, and 21.49% in Daher Aljabal, Brieh, and Daraa, respectively, compared with increases of only 8.10%, 5.03%, 4.72%, and 6.21% in DH1, BR1, DA1, and DA2. Significant differences in proline accumulation were observed between several donor cultivars and their

corresponding regenerated lines, whereas differences among regenerated lines were generally not significant. The comparatively smaller increase observed in regenerated lines may reflect their higher basal proline concentrations under non-stressed conditions.

Table 8. Effect of deficit irrigation on relative water content and proline accumulation of donor cultivars and PEG-selected regenerated lines.

Genotype	Relative water content %		Reduction Rate (%)	Proline accumulation µmol/g FW		Increase Rate (%)
	100% FC	75% FC		100% FC	75% FC	
DH	66.40d	54.32d	18.19c	112.31a	140.35a	24.97a
DH1	61.50e	59.55c	3.17a	127.84a	138.20a	8.10b
BR	73.60bc	63.40b	13.86bc	79.66b	97.27c	22.11a
BR1	71.60c	62.50b	12.71bc	99.32b	104.32b	5.03b
DA	76.67ab	68.88a	10.16ab	28.66c	34.82e	21.49a
DA1	78.57a	70.53a	10.23ab	35.58c	37.26e	4.72b
DA2	74.47abc	69.21a	7.06ab	55.27bc	58.70d	6.21b
$LSD_{0.05}$	4.111	2.774	6.418	5.958	4.502	10.48

Reduction rate (%) = [(value at 100% FC − value at 75% FC) / value at 100% FC] × 100. For proline (Table 8), the increase rate (%) = [(value at 75% FC − value at 100% FC) / value at 100% FC] × 100. Means followed by different letters within a column are significantly different at $P \leq 0.05$.

3.1.3. Phenotypic Cluster Analysis Based on Field Evaluation

The field evaluation data revealed clear variation in morphological and physiological traits between the donor cultivars and the regenerated lines derived from PEG-tolerant callus. To quantify the degree of phenotypic similarity between the regenerated lines and their corresponding donor cultivars, cluster analysis was performed based on the mean values of all studied traits under both full and deficit irrigation treatments. The analysis relied on a similarity matrix based on Euclidean distance (Table 9), and the resulting dendrogram is presented in Figure 2.

The cluster analysis distributed the seven genotypes into four distinct groups (Figure 2). The first group comprised the donor cultivar Brieh (BR) and its regenerated line BR1, which exhibited a high similarity coefficient of 92.3% (Table 9). The second group included the donor cultivar Daher Aljabal (DH) and its regenerated line DH1, with a similarity coefficient of 98.2%. The regenerated line DA2,

which was derived from Daraa callus selected at 8% PEG, formed an independent cluster (third group), showing a similarity of 79.8% with its donor cultivar Daraa. The fourth group comprised the donor cultivar Daraa (DA) and its regenerated line DA1 (selected at 6% PEG), with a similarity coefficient of 92.8%.

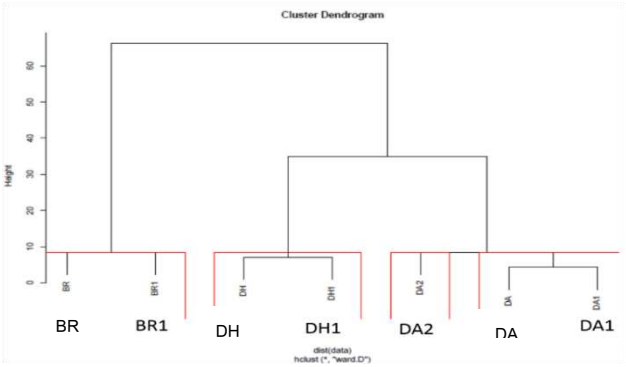


Figure 2. Cluster analysis dendrogram based on the mean values of the studied traits for the donor cultivars and the regenerated lines, two months after field planting.

Table 9. Similarity matrix between donor tomato cultivars and the regenerated lines based on the mean values of the studied traits in both full and deficit irrigation treatments together (grand mean 0.706).

Genotype	DH	DH1	BR	BR1	DA	DA1	DA2
DH	1						
DH1	**0.982**	1					
BR	0.486	0.402	1				
BR1	0.652	0.571	**0.923**	1			
DA	0.076	0.126	0.409	0.321	1		
DA1	0.113	0.045	0.429	0.351	**0.928**	1	
DA2	0.331	0.264	0.502	0.483	**0.798**	**0.839**	1

3.2. ISSR Analysis of Somaclonal Variation

3.2.1. ISSR Polymorphism Analysis

A total of 12 inter-simple sequence repeat (ISSR) primers were initially screened for amplification of genomic DNA from the donor cultivars and regenerated tomato lines. Nine primers produced usable

amplification profiles and were selected for further analysis, while the remaining three were excluded. The nine selected primers generated 39 bands, averaging 4.33 bands per primer, of which 27 were polymorphic (overall polymorphism rate of 69.23%). The mean of the individual primer-specific polymorphism percentages was 65.92% (Table 10).

Polymorphism levels varied among primers. Four primers (ISSR830, ISSR812, ISSR866, and ISSR811) exhibited 100% polymorphism, whereas ISSR nlssr3 produced no polymorphic bands. ISSRW814 generated one polymorphic band among three amplified bands, corresponding to 33.33% polymorphism. The mean polymorphism information content (PIC) across the nine primers was 0.248. ISSR812 and ISSR866 had the highest PIC values (0.408), indicating comparatively greater discriminatory potential among the analyzed genotypes, while ISSRW814 had the lowest PIC value (0.082). Overall, the ISSR analysis revealed molecular variation among the donor cultivars and regenerated lines, although the level of polymorphism and discriminatory potential differed among primers.

Table 10. ISSR primers, number of amplified bands, polymorphic bands, polymorphism percentage, and PIC values.

Primer	Total bands	Polymorphic bands	Polymorphism (%)	PIC
ISSR A830241	4	2	50	0.122
ISSR830	5	5	100	0.392
ISSR812	6	6	100	0.408
ISSR813	4	2	50	0.245
ISSRW814	3	1	33.33	0.082
ISSR866	3	3	100	0.408
ISSR8082	5	3	60	0.179
ISSR nlssr3	4	0	0	0
ISSR811	5	5	100	0.392
Total	39	27		
Mean	4.33	3	65.92	0.248

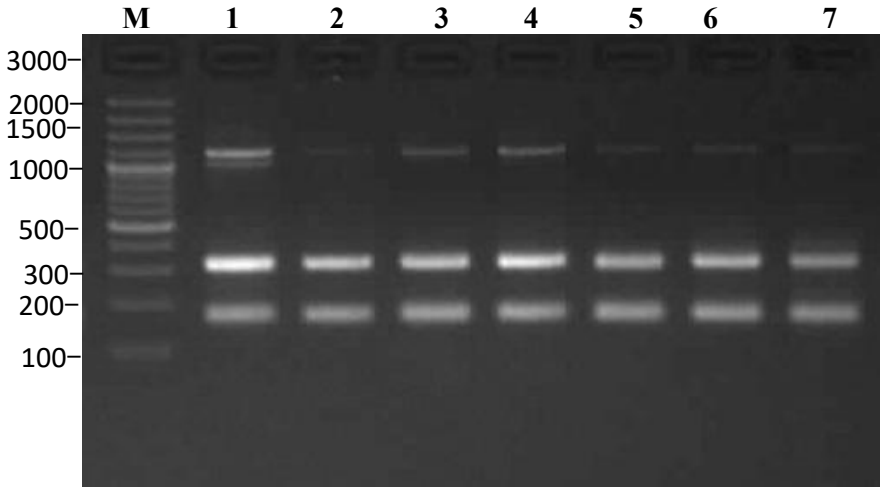


Figure 3. ISSR amplification profiles of parental tomato lines and their regenerated derivatives using primer ISSR A830241. M represents the 100-bp DNA ladder. Lanes 1–7 correspond to DA, DH, BR, DA1, DH1, BR1, and DA2, respectively.

3.2.2. Unique Bands

Amplified fragments ranged from approximately 205 to 2,500 bp. Two of the seven tomato lines were distinguished by genotype-specific bands generated using different ISSR primers (Table 11). A unique fragment of approximately 1,721 bp was detected in the donor cultivar Daraa (DA) using primer ISSRW814, whereas a unique fragment of approximately 1,357 bp was detected in regenerated line DA2 using primer ISSR8082. These unique bands may represent molecular markers associated with somaclonal variation induced during *in vitro* selection and regeneration.

Table 11. Unique bands detected in donor cultivars and regenerated lines.

Genotype	Primer	Fragment size (bp)
DA (donor cultivar)	ISSRW814	~1721
DA2 (regenerated line)	ISSR8082	~1357

3.2.3. Genetic Similarity and Cluster Analysis

Genetic relationships among the seven tomato lines were assessed using the Nei and Li similarity coefficient, and clustering was performed using the unweighted pair-group method with arithmetic mean (UPGMA). The resulting similarity matrix (Table 12) revealed varying degrees of genetic relatedness among the donor cultivars and their regenerated derivatives.

The similarity coefficient between the donor cultivars DH (Daher-Aljabal) and DA (Daraa) was 0.941, indicating a high degree of genetic similarity between these two parental lines. This close relationship suggests that these cultivars may share a common genetic background, which is relevant for interpreting the similarity patterns observed among their respective regenerated lines.

In contrast, regenerated lines exhibited substantially lower similarity to their respective donor cultivars. Specifically, the similarity coefficients were 0.541 between DH and its regenerated line DH1, 0.703 between BR (Brieh) and BR1, and 0.595 and 0.579 between DA and its regenerated lines DA1 and DA2, respectively. Among these, BR1 showed the highest similarity to its donor cultivar, suggesting that this regenerated line underwent comparatively less genomic alteration during the *in vitro* selection and regeneration process. In contrast, DH1 exhibited the greatest divergence from its parental genotype, indicating more extensive somaclonal variation.

Notably, the similarity between DA1 and DA2 (0.927) was substantially higher than the similarity of either line to their common donor DA (0.595 and 0.579, respectively). This pattern suggests that both regenerated lines derived from Daraa underwent similar genomic changes during the selection process, potentially reflecting common mechanisms of somaclonal variation or selection pressure effects.

An unexpected observation was the relatively high similarity between DH1 and DA1 (0.800), which exceeded the similarity of either regenerated line to its own donor cultivar (DH1–DH = 0.541; DA1–DA = 0.595). This counterintuitive pattern may be attributed to several factors. First, the limited number of ISSR bands (39 total) provides relatively sparse genome coverage, which may not fully capture the true genetic relationships among genotypes. Second, given the high similarity between the donor cultivars DH and DA (0.941), the regenerated lines derived from these cultivars may share ancestral genetic material that is disproportionately represented in the amplified ISSR loci. Third, the

possibility of scoring artifacts or sample handling issues cannot be entirely excluded, and verification of the band-scoring matrix is recommended.

Cluster analysis grouped the seven genotypes into four distinct clusters (Figure 4). Group I consisted exclusively of DH1, indicating substantial divergence from its donor genotype. Group II comprised two subgroups, with BR1 forming one subgroup and DA1 and DA2 grouped together in the other. Group III contained the donor cultivar BR, whereas Group IV comprised the two donor cultivars DH and DA. The clustering of DH and DA together in Group IV is consistent with their high pairwise similarity (0.941), whereas the placement of DH1 in a separate cluster reflects its substantial genetic divergence from both its donor and the other regenerated lines.

These results demonstrate that PEG-mediated *in vitro* selection and regeneration induced detectable genetic variation among the regenerated lines, with the degree of divergence varying by genotype. However, the relatively small number of ISSR markers used in this study limits the resolution of genetic relationships, and additional markers (e.g., SSR or SNP) would provide a more comprehensive assessment of the genomic changes induced during regeneration. Assessing genetic relatedness among lines is important for breeding programs, as it helps establish a broad genetic base for hybridization. The observed genetic divergence between regenerated lines and their donors, coupled with their phenotypic variation under deficit irrigation, suggests that these lines may represent useful genetic resources for tomato improvement under water-limited conditions.

Table 12. Genetic similarity matrix based on Nei and Li coefficient.

Genotype	DH	DH1	BR	BR1	DA	DA1	DA2
DH	1						
DH1	0.541	1					
BR	0.686	0.632	1				
BR1	0.611	0.612	0.703	1			
DA	0.941	0.594	0.743	0.667	1		
DA1	0.543	0.800	0.634	0.821	0.595	1	
DA2	0.579	0.732	0.615	0.800	0.579	0.927	1

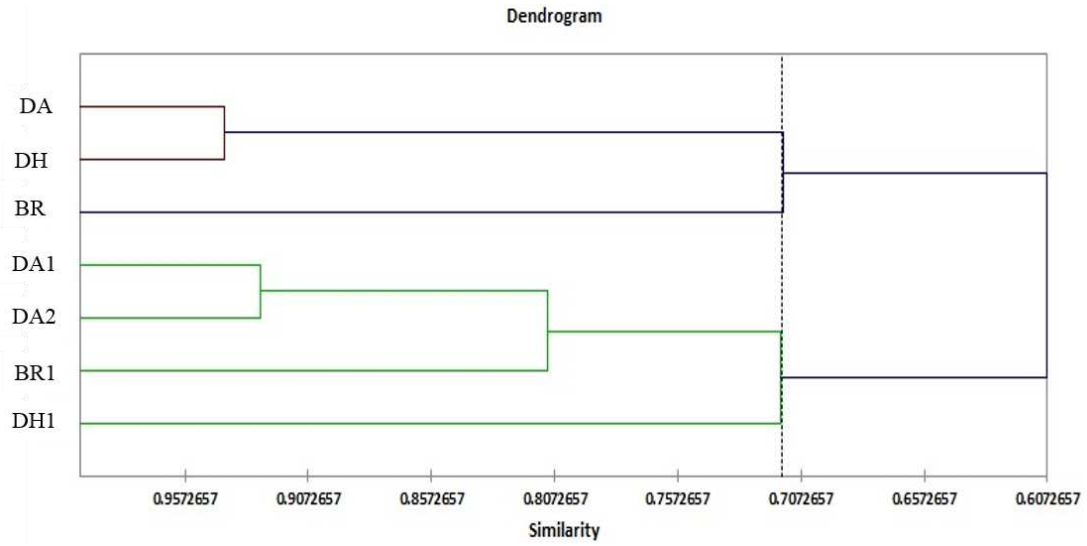


Figure 4. UPGMA dendrogram showing genetic relationships among tomato donor cultivars and PEG-selected regenerated lines based on ISSR markers.

4. Discussion

4.1. Field Performance under Deficit Irrigation

Deficit irrigation (75% field capacity) significantly affected all measured morphological and physiological traits in tomato, with the magnitude of response varying considerably among genotypes. Among the measured parameters, leaf area exhibited the greatest reduction under water deficit, followed by plant height, chlorophyll content, and relative water content, whereas dry weight, stem diameter, and branch number were comparatively less affected. These findings are consistent with previous reports documenting reduced vegetative growth and physiological impairment in tomato under deficit irrigation [13, 19, 28, 38, 17]. The pronounced sensitivity of leaf area to water deficit likely reflects the role of reduced cell expansion and division as an early adaptive response to osmotic stress, which limits transpirational water loss while preserving essential metabolic functions [37].

The reduction in relative water content (RWC) under deficit irrigation is in agreement with [21] Handayani and Watanabe (2020), who reported that drought-tolerant tomato genotypes maintained higher RWC under water stress compared with susceptible genotypes. The ability to maintain tissue hydration under water deficit is a critical determinant of drought tolerance, as it sustains photosynthetic

activity and overall plant productivity [11]. The smaller reductions in RWC observed in several regenerated lines, particularly DH1, indicate improved water status maintenance relative to their donor cultivars.

Proline accumulation increased significantly in all genotypes under deficit irrigation, consistent with the well-established role of this compatible solute in osmotic adjustment and cellular protection under water stress [20]. Proline contributes to maintaining cellular turgor, stabilizing proteins and membranes, and scavenging reactive oxygen species [22]. Notably, donor cultivars exhibited greater relative increases in proline concentration than regenerated lines, a pattern largely attributable to the higher basal proline levels already present in the regenerated lines under non-stressed conditions. However, the functional significance of this elevated constitutive proline accumulation remains unclear. It may represent a beneficial adaptation induced by *in vitro* selection, conferring enhanced osmotic tolerance through pre-existing osmotic adjustment capacity. Alternatively, it could reflect a residual stress response from the tissue culture process itself, rather than a stable adaptive trait. Distinguishing between these possibilities requires stronger mechanistic evidence. Future studies should examine proline metabolism enzymes (e.g., P5CS and proline dehydrogenase), gene expression profiles, and additional stress markers to determine whether elevated basal proline translates into improved agronomic performance under water-limited conditions.

A key finding of this study was that PEG-selected regenerated lines generally exhibited smaller reductions in growth and physiological parameters under deficit irrigation compared with their corresponding donor cultivars. *In vitro* selection under osmotic stress generated regenerants with enhanced drought tolerance. Among the regenerated lines, DA1, which was selected under a moderate PEG concentration (6%), demonstrated superior overall performance compared with DA2, which was selected under a higher PEG concentration (8%). This observation aligns with Verma et al. [38], who reported that rice regenerants selected under lower PEG concentrations exhibited superior growth

performance compared with those selected under higher PEG levels. This pattern suggests that moderate selection pressure may be optimal for recovering regenerants that combine stress adaptation with agronomic vigor, whereas excessive selection pressure may compromise overall plant fitness.

An important observation was that several regenerated lines exhibited reduced vegetative growth compared with their donor cultivars under full irrigation conditions. For example, DH1 showed significantly lower plant height (41.80 vs. 52.27 cm) and stem diameter (4.22 vs. 5.38 mm) than its donor cultivar DH, and similar reductions were observed for BR1 relative to BR. This pattern suggests a potential trade-off between stress tolerance and agronomic vigor, whereby selection for improved performance under water-limited conditions may come at the cost of reduced growth potential under optimal conditions. Such trade-offs have been documented in various crop species, where stress tolerance mechanisms often involve physiological costs, including reduced growth rates, lower photosynthetic capacity, or altered resource allocation [37]. In the present study, the elevated basal proline concentrations observed in regenerated lines may reflect a constitutive investment in osmotic adjustment that diverts resources away from vegetative growth. Similarly, other stress-associated physiological changes, such as altered hormone balances or increased antioxidant capacity, may impose metabolic costs that manifest as reduced growth under non-stressed conditions. From a breeding perspective, this trade-off has important implications. While regenerated lines such as DH1 and DA2 may offer advantages under water-limited conditions, their reduced vigor under optimal conditions may limit their direct use as commercial cultivars. However, they may serve as valuable genetic resources for crossing programs aimed at combining stress tolerance with agronomic vigor. Alternatively, the identification of regenerated lines such as DA1, which exhibited relatively good performance under both full and deficit irrigation, suggests that it may be possible to select variants that minimize the fitness costs associated with stress tolerance.

It should be noted that the field evaluation was conducted during the vegetative growth stage, and fruit yield, fruit quality, and water-use efficiency were not assessed. Therefore, the observed responses should be interpreted as preliminary evidence of potential stress adaptation rather than definitive confirmation of improved drought tolerance. Further evaluation across reproductive stages, growing seasons, and field environments is required.

4.2. Molecular Evidence of Somaclonal Variation

The ISSR analysis provided robust molecular evidence of somaclonal variation in tomato regenerants derived from PEG-selected callus cultures. The average polymorphism rate of 69.23% across the nine informative primers reflects substantial genomic variability induced during the *in vitro* regeneration process. This level of polymorphism is comparable to or higher than those reported in previous studies of somaclonal variation in tomato and other Solanaceous species [18], confirming that ISSR markers are highly effective for detecting genetic changes in tissue culture-derived plants. The ISSR technique has been reported to provide a reliable and rapid approach for detecting genetic variation across the genome [36].

The considerable variation observed among primers in their ability to detect polymorphism indicates differential genomic coverage and sensitivity of ISSR loci. Primers ISSR830, ISSR811, ISSR812, and ISSR866, which exhibited 100% polymorphism, likely target highly variable genomic regions, whereas primer ISSR nlssr3, which produced only monomorphic bands, may anneal to conserved genomic sequences. The high PIC values recorded for primers ISSR812 and ISSR866 (0.408) confirm their superior discriminatory power and utility for genetic diversity assessment in tomato. These findings underscore the importance of primer selection in molecular characterization studies, as the choice of primers can significantly influence the detection of genetic variation.

The detection of unique bands in specific genotypes provides direct molecular evidence of genetic alteration during regeneration. The unique fragment of approximately 1721 bp detected in the donor

cultivar Daraa (DA) using primer ISSRW814, and the unique fragment of approximately 1357 bp detected in regenerated line DA2 using primer ISSR8082, represent genotype-specific molecular markers associated with somaclonal variation. These unique bands may arise from mutations at primer annealing sites, insertions/deletions, or chromosomal rearrangements occurring during the callus phase and subsequent regeneration [9]. Such markers could potentially be developed into sequence-characterized amplified region (SCAR) markers for early selection of desirable somaclonal variants in breeding programs.

Cluster analysis based on the Nei and Li similarity coefficient grouped the seven genotypes into four major clusters, with DH1 forming a distinct cluster and BR1, DA1, and DA2 grouped separately from their donor cultivars. The genetic similarity coefficients between regenerated lines and their donor cultivars ranged from 0.541 (DH1 vs. DH) to 0.703 (BR1 vs. BR), indicating varying degrees of genetic divergence. The relatively higher similarity between BR and BR1 suggests that this regenerated line underwent less genomic alteration during the selection and regeneration process, whereas DH1 exhibited the greatest divergence from its parental genotype.

An interesting observation was that DH1, which was selected at the lowest PEG concentration (4%), exhibited the greatest genetic divergence from its donor cultivar (similarity = 0.541), whereas BR1, selected at 6% PEG, showed the highest similarity to its donor (0.703). This pattern suggests that the extent of somaclonal variation is not necessarily proportional to the intensity of the selection pressure applied. Several factors may explain this observation. First, the genotype of the donor cultivar likely plays a significant role in determining the frequency and nature of somaclonal variation, as different genotypes exhibit varying levels of genomic stability during tissue culture [9]. Second, the duration of the callus phase and the number of subcultures may have differed among genotypes, potentially influencing the accumulation of genetic changes. Third, the specific genomic regions amplified by the ISSR primers may have been differentially affected by the selection process in different genotypes.

These findings highlight the complexity of somaclonal variation and underscore the importance of evaluating multiple regenerated lines from each donor genotype, as the degree of genetic divergence cannot be predicted solely based on the selection pressure applied.

The molecular analysis based on binary scoring and Nei and Li similarity coefficients provides evidence that the regenerated lines are not genetically identical to their parental genotypes. However, it should be noted that ISSR markers sample only a limited portion of the genome, and the 39 bands analyzed in this study represent a relatively small fraction of the total genomic variation. Therefore, while the observed polymorphisms indicate genetic divergence, the extent of this divergence may be underestimated or overestimated depending on the genomic regions sampled. Additional markers with broader genome coverage, such as SSR or SNP markers, would provide a more comprehensive assessment of the genetic changes induced during regeneration. This finding highlights the importance of molecular screening in tissue culture and micropropagation systems to ensure genetic fidelity and to identify desirable variants. The observed somaclonal variation may arise through multiple mechanisms, including point mutations, chromosomal rearrangements, transposable element activation, and epigenetic modifications [25]. The stress imposed by PEG during *in vitro* selection may have further enhanced genomic instability, contributing to the observed genetic variation.

The findings of the present study are consistent with previous studies that successfully used ISSR markers to detect somaclonal variation associated with indirect regeneration through callus culture. Such variation has been reported in tomato [32]. The integration of molecular characterization with phenotypic evaluation, as performed in the present study, provides a comprehensive approach for assessing the potential value of somaclonal variants in crop improvement programs.

4.3. Integration of Molecular and Phenotypic Evidence

The integration of ISSR-based molecular characterization with field evaluation provides a comprehensive understanding of the effects of PEG-mediated *in vitro* selection on tomato regenerants.

The molecular evidence of genetic divergence between regenerated lines and their donor cultivars corroborates the observed phenotypic differences under both full and deficit irrigation conditions. Regenerated lines that exhibited greater genetic divergence from their donor cultivars, such as DH1, also demonstrated distinct physiological characteristics, including elevated proline accumulation and improved performance under water deficit.

The persistence of stress-associated physiological adaptations in regenerated lines, as evidenced by elevated basal proline concentrations and improved performance under deficit irrigation, suggests that the *in vitro* selection process induced stable genetic and/or epigenetic changes that are expressed under field conditions. This finding supports the utility of PEG-mediated *in vitro* selection as a strategy for generating drought-tolerant tomato genotypes with potential value for breeding programs.

However, it is important to note that the reduced vegetative growth observed in several regenerated lines under full irrigation conditions may represent a trade-off between stress tolerance and agronomic vigor. This trade-off has been documented in various crop species, where selection for stress tolerance often results in reduced growth under optimal conditions [37] (Tardieu et al., 2014). The identification of regenerated lines that combine stress tolerance with acceptable agronomic performance, such as DA1, is therefore of particular interest for breeding applications.

5. Conclusions

This study integrated field evaluation with ISSR-based molecular characterization to assess the phenotypic performance and genetic variation of PEG-selected tomato regenerants relative to their donor cultivars under full and deficit irrigation. The results revealed genotype-dependent responses to water deficit, with several regenerated lines exhibiting smaller reductions in key morphological and physiological traits compared with their corresponding donors. However, these responses were not

uniform across genotypes or traits, underscoring the importance of evaluating multiple regenerants rather than assuming that stronger PEG selection necessarily produces superior plants.

ISSR analysis detected substantial molecular polymorphism among the evaluated genotypes, with an overall polymorphism rate of 69.23% and genotype-specific bands in the donor cultivar Daraa and the regenerated line DA2. Genetic similarity coefficients between regenerated lines and their donors ranged from 0.541 to 0.703, indicating varying degrees of somaclonal variation. Nevertheless, the association between molecular divergence and stress-related performance remains to be established, and the limited number of markers used warrants caution in interpreting the extent of genetic divergence. Similarly, the elevated basal proline concentrations observed in regenerated lines under non-stressed conditions require further investigation to determine whether they represent stable adaptive traits or residual stress responses from the regeneration process.

PEG-mediated *in vitro* selection followed by regeneration may provide a useful approach for generating tomato material with potentially favorable responses to limited water availability. The evaluated lines should be regarded as candidates for further testing rather than confirmed drought-tolerant cultivars. Multi-season and multi-location trials incorporating reproductive performance, fruit yield, fruit quality, and water-use efficiency are needed to determine their agronomic value and suitability for tomato breeding programs.

From a practical perspective, the regenerated lines identified in this study—particularly DH1 and DA2—represent promising genetic resources for tomato breeding programs targeting water-limited environments. Their potential value lies not only in their direct use as improved lines but also as parental material for hybridization with locally adapted cultivars, thereby combining stress tolerance with desirable agronomic traits. However, the observed trade-off between stress tolerance and vegetative vigor under optimal conditions should be carefully considered when selecting lines for breeding applications.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare no competing interests.

Funding

There was no funding.

Authors' contributions

O.A.: Conceptualization, methodology, investigation, resources, data curation, formal analysis, validation, writing—original draft preparation, writing—review and editing, visualization, project administration. H.A.: Methodology, investigation, formal analysis, validation, data curation, writing—review and editing. W.M.: Conceptualization, methodology, investigation, writing—review and editing, supervision. All authors read and approved the final manuscript.

Acknowledgements

Not applicable.

Abbreviations

Abbreviation	Full Form
ANOVA	Analysis of Variance
BAP	6-Benzylaminopurine
CTAB	Cetyltrimethylammonium Bromide
DNA	Deoxy Ribonucleic Acid
dNTPs	Deoxynucleotide Triphosphates
EDTA	Ethylenediaminetetraacetic Acid
FC	Field Capacity
GA_3	Gibberellic Acid
GCSAR	General Commission for Scientific Agricultural Research
IBA	Indole-3-Butyric Acid
ISSR	Inter-Simple Sequence Repeat
LSD	Least Significant Difference
MS	Murashige and Skoog
NAA	Naphthaleneacetic Acid
NCBT	National Commission for Biotechnology
PCR	Polymerase Chain Reaction
PEG	Polyethylene Glycol
PIC	Polymorphism Information Content
RCBD	Randomized Complete Block Design
RWC	Relative Water Content
SCAR	Sequence-Characterized Amplified Region

SNP	Single Nucleotide Polymorphism
SSR	Simple Sequence Repeat
TBE	Tris-Borate-EDTA
UPGMA	Unweighted Pair Group Method with Arithmetic Mean

References

1. Akram S, Saleem Y, Khan AR. et al. Identification of Drought-Tolerant Tomato Genotypes Using Multi-trait Index at Early Growth Stage. J Soil Sci Plant Nutr 24, 2456–2468 (2024). https://doi.org/10.1007/s42729-024-01665-5
2. Alnaddaf O, Mohsen W, Obaid H. Callus Induction and Regeneration Responses of Four Local Tomato Varieties (*Solanum Lycopersicum* L.). Arab J Arid Environ. 2022;15:298–316
3. Alnaddaf O, Mohsen W, Alsafadi F. The RESPONSE of Eight Pure Tomato Lines (*Lycopersicon esculentum*) for Drought Stress Induced by Polyethylene Glycol *in vitro*. Syrian J Agric Res. 2024;12(4)
4. Alnaddaf O, Mohsen W, Al-Tawaha AR et al. Physiological responses of tomato callus and regenerated plants under PEG induced stress during *in vitro* selection for drought tolerance. BMC Plant Biol. 2026; 26:1239. https://doi.org/10.1186/ s12870-026-08989-7
5. Amier N, Farid M, Dermawan R , Adnan ,Anshori MF, Hendra J. Yield assessment of F6 generation tomato lines under irrigated and drought environments based on stress tolerance index. Reproduction and Breeding 5 (2025) 214–221
6. Amiteye S. Basic concepts and methodologies of DNA marker systems in plant molecular breeding. Heliyon. 2021 30;7(10):e08093. doi: 10.1016/j.heliyon.2021.e08093. PMID: 34765757; PMCID: PMC8569399.

7. Anderson JA, Churchill GA, Autrique JE, Tanksley SD, and Sorrells ME. Optimizing parental selection for genetic linkage maps. Genome. 1993; 36 (1):181–186.
8. Bates LS, Waldren RP, Teare ID. Determination of free proline. Plant Soil. 1973;39:205–207
9. Bednarek PT, Orłowska R. Plant tissue culture environment as a switch of genetic changes. Plant Cell Tissue Organ Cult. 2020;140:245–257
10. Birsin MA, Ozgen M. Callus induction in triticale. Cell Mol Biol Lett. 2004;9:353–361
11. Blum A. (2010): Plant breeding for water-limited environments. Springer, New York
12. Chadipiralla K, Gayathri P, Rajani V, Reddy PVB. Plant Tissue Culture and Crop Improvement. In: Roychowdhury, R., Choudhury, S., Hasanuzzaman, M., Srivastava, S. (eds) Sustainable Agriculture in the Era of Climate Change. Springer, Cham. 2020. https://doi.org/10.1007/978-3-030-45669-6_18
13. Chand J, Hewa G, Hassanli A. and Myers B. Evaluation of Deficit Irrigation and Water Quality on Production and Water Productivity of Tomato in Greenhouse. Agriculture. 2020; 10, 297.
14. Cepkova PH, Mrhalova H. and Eliasova K. 2019. Assessment of somaclonal variation in indirect morphogenesis-derived plants of *Arracacia xanthorrhiza*. Pesquisa Agropecuária Brasileira,2019; v.54, e0030.
15. El-Yacoubi H, Ayolié K, Rochdi A. Salt tolerance in citrange callus. Int J Agric Biol. 2010;12:187–193.
16. Erdinc C, Ekincialp A, Yildiz M, Kabay T, Turkmen O. and Sensoy S. Molecular genetic diversity in lakevan basin melon (*Cucumis melo* L.) based on RAPD and ISSR markers. Yyü Tar Bil Derg. Journal of Agricultural Sciences. 2013; 23(3): 264- 270.
17. Fawzy ZF, Ragab ME, Arafa YE, Sawan OM and El-Sawy SM. Effect of Irrigation Systems on Vegetative Growth, Fruit Yield, Quality and Irrigation Water Use Efficiency of Tomato Plants

(*Solanum lycopersicum* L.) Grown under Water Stress Conditions. Acta Scientific Agriculture. 2019; 3(4):172-183.

18. Gemmill CEC and Grierson ERP. Inter-Simple Sequence Repeats (ISSR), Microsatellite-Primed Genomic Profiling Using Universal Primers. In: Besse, P. (eds) Molecular Plant Taxonomy. Methods in Molecular Biology.2021., vol 2222. Humana, New York, NY. https://doi.org/10.1007/978-1-0716-0997-2_14
19. Ghannem A, Ben Aissa I. and Majdoub,R. Effects of regulated deficit irrigation applied at different growth stages of greenhouse grown tomato on substrate moisture, yield, fruit quality, and physiological traits. Environ Sci Pollut Res. 2021; 28, 46553–46564. https://doi.org/10.1007/s11356-020-10407-w
20. Guo HM, Zhang YQ, Wan FH and Cheng HM. Agrobacterium-mediated transformation of Eupatorium adenophorum. Plant Cell Tissue and Organ Culture. 2010; 103:417–422.
21. Handayani T, and Watanabe K. The combination of drought and heat stress has a greater effect on potato plants than single stresses. Plant, Soil and Environment. 2020; 66 (4): 175–182.
22. Hayat S, Hayat Q, Alyemeni MN, et al. Role of proline under stress. Plant Signal Behav. 2012;7(11):1456–1466
23. Leva AR, Petruccelli R. and Rinaldi LMR. Somaclonal variation in tissue culture: a case study with olive. Chapter 7. In Recent Advances in Plant *In Vitro* Culture. 2012; Pp:123-150
24. Lichtenthaler HK. (1987). Chlorophylls and carotenoids: Pigments of photosynthetic biomembranes. Methods in Enzymology, 148, 350–382. https://doi.org/10.1016/0076-6879(87)48036-1
25. Liu M, Zhao G, Huang X, Pan T, Chen W, Qu M, Ouyang B, Yu M and Shabala S. Candidate regulators of drought stress in tomato revealed by comparative transcriptomic and proteomic analyses. Front. Plant Sci. 2023; 14:1282718. doi: 10.3389/fpls.2023.1282718

26. Long Y, Yang Y, Pan G, Shen Y. New Insights Into Tissue Culture Plant-Regeneration Mechanisms. Front Plant Sci. 2022 Jun 30;13:926752. doi: 10.3389/fpls.2022.926752. PMID: 35845646; PMCID: PMC9280033.
27. Lutts S, Almansouri M, Kinet JM. Salinity and water stress effects in wheat callus. Plant Sci. 2004;167:9–18.
28. Mesquita M, Pureza Machado A, Dos Santos De Paula A, Da Silva MV, Fonseca De Oliveira H, Battisti R. and Abadia Dos Reis N. Assessing the Effects of Deficit Irrigation Techniques on Yield and Water Productivity of Processing Tomato, Chemical Engineering Transactions. 2019; 75: 181-186.
29. Murashige T, Skoog F. A revised medium for tissue culture. Physiol Plant. 1962;15(3):473–497
30. Nei M. and Li WH. Mathematical model for studying genetic variation in terms of restriction endonucleases. Proc. Natl. Acad. Sci. 1979; 76:5269–5273.
31. Pithiya MB, Sharma SK, Sharma M, Sharma M, Kotwal N. Advancements and Challenges in Plant Tissue Culture: a Comprehensive Overview. Journal of Plant Biota. 2022 https://doi.org/10.51470/JPB.2022.1.1.12
32. Rzepka-Plevneš D, Kulpa D, Smolik M. and Gáówka M. Somaclonal variation in tomato L. pennelli and L. peruvianum f. glandulosum characterized in respect to salt tolerance. Journal of Food, Agriculture and Environment. 2007; Vol.5(2): 194-201.
33. Soares DMM, Sattler MC, Ferreira MF, Da S. and Praça-Fontes MM. Assessment of genetic stability in three generations of *in vitro* propagated Jatropha curcas L. plantlets using ISSR markers. Tropical Plant Biology, 2016; Vol.9, p.229-238.
34. Sharma P, Kurubar AR, Tembhurne BV, Patil S. *In vitro* screening of chilli genotypes for drought tolerance. J Hortic Sci. 2024;19(1). https://doi.org/10.24154/jhs.v19i1.1882

35. Shehzad MT, Ghazanfar H, Hussain M, Farooqi ZuR, Hussain MM, Bashir H. The Influence of Drought Stress on Tomatoes: A Brief Understanding. In: Chaudhry UK, Öztürk ZN, Gökçe AF. (eds) Drought Stress. Springer, Cham. https://doi.org/10.1007/978-3-031-80610-0_8
36. Solano MCP, Ruíz JS, Arnao MTG, Castro OC, Tovar MEG and Bello JJB. Evaluation of *in vitro* shoot multiplication and ISSR marker based assessment of somaclonal variants at different subcultures of vanilla (*Vanilla planifolia* Jacks). Physiology and Molecular Biology of Plants. 2019; 25: 561-567.
37. Tardieu F, Simonneau T. and Muller B. The physiological basis of drought tolerance in crop plants: a scenario-dependent probabilistic approach. Annual Review of Plant Biology. 2018; v69, pp. 733–759.
38. Verma D, Ansar MW, Agrawal GK, Rakwal R, Shukla A. and Tuteja N. *In vitro* selection and field responses of somaclonal variant plants of rice cv PR113 for drought tolerance. Plant Signaling and Behavior. 2013; 8 (4): e23519.
39. Wahb-Allah MA, Alsadon AA. and Ibrahim AA. Drought tolerance of several tomato genotypes under greenhouse conditions World Applied Sciences Journal. 2021; 15 (7): 933-940.
40. Wijerathna-Yapa A, Hiti-Bandaralage J. Tissue Culture—A Sustainable Approach to Explore Plant Stresses. Life. 2023; 13(3):780. https://doi.org/10.3390/life13030780

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryFigureFullllengthunprocessedagarosegelimageforISSRprimerA830241correspondingtoFigure3inthemainmanuscript..docx