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EVALUATION OF DROUGHT TOLERANCE IN GROUNDNUT (*ARACHIS HYPOGAEA* L.) VARIETIES UNDER PEG-INDUCED STRESS USING PHYSIOLOGICAL AND BIOCHEMICAL MARKERS

G.D. Guna, Darshna G. Hirpara, Disha D. Savaliya and H. P. Gajera*
Department of Biotechnology, College of Agriculture, Junagadh Agricultural University, Junagadh, Gujarat, India – 362001
*Corresponding author E-mail: harsukhgajera@yahoo.com
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ABSTRACT

Drought stress significantly hampers physiological and biochemical processes in groundnut (*Arachis hypogaea* L.) at the early stages of seed germination and seedling growth. A study was conducted to evaluate the drought tolerance of ten groundnut varieties under polyethylene glycol (PEG-6000) induced drought stress (10%–25%) at 15 days after sowing (DAS) in a controlled environment. Key physiological (germination %, moisture %, relative water content, membrane stability index, drought tolerance index, root and shoot length) and biochemical parameters (chlorophyll a, chlorophyll b, total chlorophyll, free proline, glycine betaine, and lipid peroxidation) were measured. Drought stress significantly reduced germination, chlorophyll content, and water status, while increasing oxidative damage and osmolyte accumulation. Variety GJG-31 showed superior drought tolerance, reflected by higher germination (80.67%), RWC (90.52%), MSI (71.18%), proline (0.281 mg/g FW), and glycine betaine (0.247 mg/g FW) under 25% PEG stress. In contrast, variety GG-7 exhibited the most sensitivity. Correlation analysis revealed strong positive associations between DTI and RWC (r = 0.8857), MSI (r = 0.8380), and germination % (r = 0.7802). Lipid peroxidation showed significant negative correlations with most stress-tolerance indicators, particularly proline (r = –0.8559), RWC (r = –0.7173), and total chlorophyll (r = –0.7118). These findings highlight the effectiveness of physiological and biochemical markers in assessing drought tolerance in groundnut genotypes and identify GJG-31 as a promising variety for drought-prone regions.
***Keywords*:** Groundnut, PEG induced stress, Lipid Peroxidation, Variety screening, physiological changes, Biochemical markers.

Introduction

Groundnut (*Arachis hypogaea* L.), also known as peanut, is an economically important annual leguminous oilseed crop originating from South America. It plays a dual role in human nutrition and industrial applications due to its high oil (44–50%) and protein (30%) content. Groundnut is consumed in a variety of forms including roasted nuts, confections, peanut butter, and is a primary source of edible oil. The oil derived from groundnut is highly valued for its balanced fatty acid profile, comprising oleic acid (40–50%) and linoleic acid (25–35%) (Mathur & Khan, 1997), making it a staple in culinary practices, especially in the form of refined oil and vanaspati ghee.

In India, oilseeds represent the second most significant group of agricultural crops after cereals, occupying around 14% of the gross cropped area and contributing approximately 10% of the total value of agricultural production. However, groundnut cultivation is frequently challenged by water scarcity, especially in rainfed regions, which adversely affects crop productivity.

Drought stress, a critical abiotic stress, impairs several physiological and biochemical processes in plants. It leads to reduced relative water content

(RWC), closure of stomata, reduced turgor pressure, and overall decline in growth and yield. Morphological and physiological disruptions due to drought include decreased photosynthetic activity, respiration rate, membrane integrity, and nutrient transport (Toker & Cagirgan, 1998).

To mimic drought conditions under controlled experimental setups, polyethylene glycol (PEG) is often used. PEG creates osmotic stress that simulates water deficit, allowing for the assessment of drought tolerance in plants without inducing ion toxicity. In response to such stress, plants often accumulate compatible solutes like proline and glycine betaine and exhibit increased levels of oxidative stress indicators such as malondialdehyde (MDA), a marker of lipid peroxidation. Understanding how different groundnut varieties respond to PEG-induced drought stress at the germination and seedling stage is essential for identifying tolerant genotypes. This study aims to assess changes in physiological and biochemical parameters under PEG-induced drought stress, with a particular focus on identifying genotypic variation in stress tolerance.

Material and Methods

Experimental Design and Treatments

Ten groundnut (*Arachis hypogaea* L.) varieties GG-6, GG-2, GG-7, GJG-9, GG-11, GG-21, GJG-22, GJG-31, GJG-32 and GJG-33 were sourced from the Main Oilseeds Research Station, Junagadh. Seeds were germinated in petri plates under five drought treatments simulated using polyethylene glycol (PEG-6000) at concentrations of 0% (T0 – Control), 10% (T1), 15% (T2), 20% (T3), and 25% (T4). The study followed a Factorial Completely Randomized Design (FCRD) to evaluate the individual and interactive effects of genotype and drought stress on physiological and biochemical traits.

Physiological and Biochemical Assessments

Germination Percentage: Ten seeds per treatment were placed in petri plates, and germination was recorded on the 5th day (I.S.T.A., 1976).

Relative Water Content (RWC): Fresh leaf samples were weighed, soaked in distilled water for 4 hours, then reweighed (turgid weight). After oven-drying at 84°C for 5 hours, dry weight was recorded (Weatherley, 1962).

$$\text{RWC}(\%) = \frac{\text{Fresh wt.} - \text{Dry wt.}}{\text{Turgid wt.} - \text{Dry wt.}} \times 100$$

Drought Tolerance Index (DTI): Root and shoot lengths were measured at 15 days after sowing, and DTI was calculated as per Rahman *et al*. (2008).

Moisture Content (%): Leaf samples were oven-dried at 105°C for 5 hours. Moisture percentage was calculated based on weight loss (A.O.A.C., 1980).

Chlorophyll Content: Chlorophyll a, b, and total chlorophyll were estimated by extracting 0.1 g of fresh leaf tissue in 80% chilled acetone. Absorbance was measured at 645 nm and 663 nm (Arnon, 1949).

Membrane Stability Index (MSI): MSI was assessed by measuring electrical conductivity of leaf tissues before and after heating (Sairam *et al*., 1997).

Free Proline Content: Estimated using the method of Bates *et al*. (1973), 0.1 g of leaf tissue was homogenized in 3% sulfosalicylic acid. After reaction with acid ninhydrin and extraction with toluene, absorbance was read at 520 nm. Results were expressed as $mg \cdot g^{-1}$ fresh weight.

Glycine Betaine Content: Following Hendawey (2015), 0.5 g of fresh leaf was extracted in water for 16 hours at 25°C. After filtration, the extract was reacted with potassium iodide–iodine reagent and extracted into dichloroethane. Absorbance was recorded at 365 nm, and glycine betaine was quantified using standard curves.

Lipid Peroxidation (MDA Content): Measured as malondialdehyde (MDA) content via thiobarbituric acid (TBA) reaction (Heath & Packer, 1968). Leaf tissue (1 g) was homogenized in TCA with PVP, and the supernatant was reacted with TBA. After heating and centrifugation, absorbance was measured at 532 nm and 600 nm. MDA concentration was expressed in $nmol \cdot g^{-1}$ fresh weight using an extinction coefficient of 155 $mM^{-1}cm^{-1}$

Results and Discussion

Germination Percentage

Germination percentage was assessed five days after sowing (DAS) in petri plates treated with varying concentrations of PEG-6000 (0%, 10%, 15%, 20%, and 25%), simulating drought stress. Ten groundnut varieties were tested, and results are summarized in Table 1.The analysis revealed a significant effect of both variety and PEG concentration on germination. Among the varieties, GJG-31 (V8) exhibited the highest average germination percentage (90.40%), whereas GG-7 (V3) showed the lowest (80.66%). Similarly, treatment effects varied significantly, with T0 (control) showing the highest mean germination (99.93%) and T4 (25% PEG) the lowest (70.87%).The interaction between variety and treatment was also significant. Under non-stress conditions (T0), nearly all varieties achieved 100% germination, except GG-7 (99.33%). However, with increasing PEG concentrations, germination declined across all varieties. Notably, GG-7 at T4 (25% PEG) recorded the lowest germination (60.67%), while GJG-31 under T4 maintained relatively higher germination (80.67%), suggesting its greater tolerance to drought stress.

These findings align with those of Guo *et al.* (2024), who observed significant reductions in germination under PEG-6000 concentrations above 15%. While mild PEG stress (5–10%) had negligible or even stimulatory effects, germination dropped sharply at 20% PEG. The lowest germination energy and germination index were also reported at 20% PEG, indicating adverse physiological impacts at higher osmotic stress levels. Similarly, Xu *et al.* (2015) reported enhanced germination of *Apocynumvenetum* under mild drought (5–10% PEG), but significant inhibition at higher concentrations. The onset of germination was also delayed under severe stress.In groundnut, Kokkanti *et al.* (2019) found that germination rates declined with decreasing osmotic potential. Seeds under moderate PEG levels (-2 to -4 bars) showed better vigor, but germination was severely inhibited at -10 to -12 bars. No germination occurred at -14 bars, except in the drought-tolerant Dharani variety, which still showed 20% germination highlighting genotypic variation under stress. Overall, the results of the present study confirm that PEG-induced osmotic stress significantly affects germination, and GJG-31 demonstrated superior drought tolerance, maintaining higher germination rates under all stress levels.

Relative Water Content (RWC)

Relative water content (RWC) was evaluated at 15 days after sowing (DAS) across ten groundnut varieties subjected to varying levels of PEG-induced drought stress (0% to 25%). The data, summarized in Table 2, highlights the significant effects of both genotype and treatment on leaf water status.

Among varieties, GJG-31 (V8) recorded the highest RWC (90.53%), statistically at par with GJG-32 (V9) (86.37%). In contrast, the lowest RWC (78.32%) was observed in GG-7 (V3), indicating its higher susceptibility to water stress.

Treatment effects showed a clear decline in RWC with increasing PEG concentration. The control treatment (T0, distilled water) had the highest mean RWC (89.36%), whereas the 25% PEG treatment (T4) recorded the lowest (77.27%). This trend confirms that increasing osmotic stress reduces cellular water retention.

Significant interaction effects were also noted between variety and treatment. Under severe stress (25% PEG), GJG-31 (V8) retained the highest RWC (83.70%), whereas GG-11 (V5) showed the lowest (71.90%). This suggests that GJG-31 possesses superior water retention mechanisms under drought, potentially due to better osmotic adjustment or membrane stability.

These findings align with prior studies. Kalariya *et al.* (2013) reported a reduction in RWC from 92% to 88% and 91% to 84% under water deficit conditions across groundnut growth stages. In a follow-up study, Kalariya *et al.* (2015) observed a progressive RWC decline with increased drought duration mean values dropped from 92% to 61% over 60 days in Spanish groundnut cultivars.

Bhattacharjee *et al.* (2023) demonstrated similar RWC reductions in rice under PEG stress, with RWC declining from 86% to as low as 62%, depending on the variety and PEG level. Notably, drought-tolerant genotypes like Sahbhagi Dhan maintained higher RWC than susceptible ones. Asati *et al.* (2024) also reported decreased RWC in chickpea under drought, with mean values dropping from 52.55% (normal) to 47.61% (stress). Tolerant varieties like SAGL152278 retained more water, while susceptible lines showed greater declines.Further, Ishfaq *et al.* (2024) found that drought stress reduced RWC in wheat by up to 55%, along with reductions in water potential and stomatal conductance.

Overall, the results of this study underscore that RWC is a reliable physiological marker of drought tolerance, and GJG-31 consistently maintained higher water status, indicating its superior adaptability to drought conditions.

Drought Tolerance Index (DTI)

Drought Tolerance Index (DTI) was assessed in ten groundnut varieties grown in petri dishes under controlled laboratory conditions, based on root and shoot lengths measured at 15 days after sowing (DAS). The data (Table 3) demonstrated statistically significant differences among varieties and treatments.

Among the genotypes, GJG-31 (V8) recorded the highest mean DTI (80.53%), indicating its superior drought resilience, while GG-7 (V3) showed the lowest DTI (51.49%), reflecting higher sensitivity to water deficit. Treatment-wise, a decreasing trend in DTI was observed with increasing PEG-induced drought stress. The control treatment (T0) had the highest mean DTI

(98.13%), while the T4 treatment (25% PEG) recorded the lowest (50.52%). This confirms the negative impact of osmotic stress on seedling growth and drought adaptation.

The interaction effect of variety and treatment was also significant. Under the most severe stress (25% PEG), GJG-31 (V8T4) maintained the highest DTI (65.67), whereas GG-7 (V3T4) had the lowest DTI (36.84). These results further affirm that GJG-31 possesses enhanced physiological mechanisms to maintain growth under drought.

These observations are consistent with findings by Sun *et al*. (2023), who reported that the Stress Tolerance Index (STI) is closely correlated with yield under drought, identifying high-STI genotype like CQJ-5 as drought-tolerant. Similarly, Sintaha *et al*. (2022) observed that soybean varieties exposed to repeated drought stress had lower Drought Stress Index (DSI) and higher survival rates, suggesting enhanced resilience through adaptation mechanisms.

DTI proved to be an effective indicator for evaluating drought tolerance at the seedling stage, with GJG-31 emerging as the most promising variety for drought-prone environments due to its consistent performance across all stress levels.

Moisture Percentage

Moisture percentage in groundnut leaves was measured at 15 days after sowing (DAS) in ten varieties grown under varying levels of PEG-induced drought stress (10% to 25%) alongside a control (T0). The data (Table 4) revealed significant differences among varieties and treatments.

Among genotypes, GJG-31 (V8) recorded the highest mean moisture content (77.95%), closely followed by GG-2 (V2) with 76.28%. In contrast, GG-7 (V3) exhibited the lowest moisture content (49.53%), indicating a greater sensitivity to drought.

Treatment effects showed a clear decline in moisture content with increasing PEG concentrations. The control (T0) recorded the highest mean moisture (86.63%), while the T4 treatment (25% PEG) had the lowest (59.66%). This reflects the expected moisture loss under osmotic stress conditions.

The interaction between variety and treatment was statistically significant. Under the most severe drought stress (T4), GJG-31 (V8T4) retained the highest moisture content (63.14%), whereas GG-7 (V3T4) dropped to the lowest (37.35%). This suggests that GJG-31 exhibits superior water retention capacity under stress, likely due to better osmotic adjustment or cellular water conservation mechanisms.

These findings are in line with Swathi *et al*. (2024), who emphasized the importance of controlling moisture in groundnut to reduce aflatoxin contamination, particularly noting elevated risk at 14% moisture. Similarly, Venkata Reddy and Mathew (2022) reported that increasing seed moisture affects physical properties such as size and density, impacting both storage and processing outcomes. Thus, maintaining optimal moisture is critical not only for drought resilience but also for post-harvest quality.

Chlorophyll Content

Chlorophyll a Content

Chlorophyll a content was measured at 15 DAS in groundnut seedlings subjected to varying PEG-induced drought stress (10%–25%) and control (T0). The results (Table 5) indicate significant differences among varieties and treatments.

The highest mean chlorophyll a content was observed in GJG-31 (V8) with 0.087 mg/g FW, while the lowest was found in GG-7 (V3) with 0.055 mg/g FW. Treatment-wise, the control (T0) had the highest mean value (0.080 mg/g FW), which progressively declined under increasing PEG concentrations, with the lowest mean (0.055 mg/g FW) recorded at 25% PEG (T4).

The interaction effect of variety and treatment was statistically significant. Under T4, GJG-31 retained the highest chlorophyll a level (0.070 mg/g FW), while GG-7 exhibited the lowest (0.037 mg/g FW), highlighting the relative drought sensitivity of GG-7 and tolerance of GJG-31.

These observations are supported by Batool *et al*. (2020), who reported that drought stress led to a 39–67% reduction in chlorophyll a in potato varieties. Similarly, Asati *et al*. (2024) found a mean reduction of 0.074-fold in chickpea genotypes under drought, with the highest decreases observed in sensitive genotypes and minimal losses in drought-tolerant lines.

Chlorophyll b Content

Chlorophyll b content, also assessed at 15 DAS, varied significantly across both groundnut genotypes and PEG treatments (Table 6). The highest mean value was recorded in GJG-31 (V8) (0.079 mg/g FW), while GG-7 (V3) showed the lowest (0.047 mg/g FW).

Among treatments, the control (T0) showed the maximum chlorophyll b content (0.071 mg/g FW), whereas drought stress (especially at 25% PEG) caused a marked reduction, with a mean value of 0.047 mg/g FW.

The variety × treatment interaction was also significant. Under T4, GG-7 recorded the lowest chlorophyll b content (0.029 mg/g FW), whereas GJG-31 maintained significantly higher levels (0.063 mg/g FW), further demonstrating its superior drought resilience.

These findings are consistent with Batool *et al.* (2020), who noted a 35–69% decline in chlorophyll b under drought in potato cultivars. Similarly, Asati *et al.* (2024) reported chlorophyll b reductions of 0.02–0.17-fold in chickpea, with drought-sensitive genotypes being the most affected.

Total Chlorophyll Content

Total chlorophyll content was measured in leaves of various groundnut varieties subjected to PEG-induced drought stress (10% to 25%) at 15 DAS. The data (Table 7) demonstrate significant variation among varieties and treatments.

The highest mean total chlorophyll content was recorded in GJG-31 (V8) with 0.167 mg/g FW, whereas the lowest was observed in GG-7 (V3) at 0.103 mg/g FW. Across treatments, the control irrigated with distilled water (T0) exhibited the highest total chlorophyll content (0.151 mg/g FW), which declined significantly as PEG concentration increased, with values ranging between 0.146 and 0.102 mg/g FW.

The interaction effect between variety and PEG treatment was significant. Under severe stress (25% PEG, T4), the lowest total chlorophyll content was recorded in GG-7 (0.067 mg/g FW), while GJG-31 maintained a significantly higher content (0.134 mg/g FW), highlighting its relative drought tolerance.

Chlorophyll plays a crucial role in photosynthesis by facilitating light absorption and energy conversion. Its degradation under drought stress directly impacts plant physiological performance and drought resistance (Yang *et al*., 2023). Similar patterns of chlorophyll decline under drought have been reported in various species: Yan *et al.* (2024) observed an initial increase followed by a decline in chlorophyll a, b, and total content in blue honeysuckle; Qi *et al*. (2023) noted chlorophyll peaked before declining with increased PEG levels in drought-stressed plants; and Razi *et al.* (2021) found a progressive chlorophyll reduction from day 5 to day 10 under drought.

Furthermore, Huang *et al*. (2022) reported a rapid decrease in total chlorophyll and its components in Artemisia selengensis under drought stress. Ishfaq *et al*. (2024) showed a 25–26% reduction in chlorophyll a and b in wheat, with drought-tolerant varieties maintaining higher chlorophyll levels compared to sensitive ones. Li *et al*. (2024) demonstrated significant declines in chlorophyll components under combined cold and drought stress in Poa annua germplasms, with differential reductions indicating varied stress sensitivity.

Membrane Stability Index (MSI)

The membrane stability index (MSI) was assessed in leaves of various groundnut varieties exposed to different concentrations of PEG-induced drought stress (10% to 25%, treatments T1 to T4) alongside a control (T0, irrigated with distilled water), at 15 days after sowing (DAS). The results are summarized in Table 8.

Analysis of the data indicates that groundnut varieties differed significantly in their MSI at the seedling stage. The variety GJG-31 (V8) recorded the highest mean MSI of 71.18%, while the lowest MSI of 45.90% was observed in GG-7 (V3). Across treatments, MSI varied significantly from 68.07% to 47.75%, with the control treatment (T0) showing the highest mean MSI value of 74.73%. Increasing PEG concentration, simulating drought stress, led to a consistent decline in MSI compared to the control.

The interaction between variety and PEG treatment was statistically significant. At the highest PEG concentration (25%, T4), the highest MSI was observed in GJG-31 (50.96%), whereas the lowest MSI was noted in GG-7 (38.04%), highlighting varietal differences in membrane stability under drought conditions.

Membrane Stability Index is a critical physiological marker used in drought stress research to evaluate cell membrane integrity. It measures electrolyte leakage caused by membrane damage, where higher MSI values indicate stronger membrane integrity and greater drought tolerance. The MSI reflects the degree of membrane injury and the plant's capacity to withstand drought-induced cellular damage.

These findings align with previous studies. Sakya *et al.* (2018) reported a reduction in MSI of 13.5% in roots and 16.7% in leaves of tomato plants under drought compared to well-watered controls, underscoring how water stress disrupts membrane permeability. Similarly, Abid *et al.* (2018) found significant MSI reductions under severe drought stress, especially at the jointing stage, with tolerant plants maintaining higher MSI and lower membrane injury (MI) than sensitive ones. Recovery patterns of MSI and MI after re-watering also differed depending on stress severity.

Dwivedi *et al.* (2018) observed the highest leaf MSI at the pre-anthesis stage (82.9%) in wheat, which declined through anthesis and post-anthesis stages. Application of plant growth regulators (PGRs) improved MSI by 2% under control and up to 5% under drought stress, with the most significant improvements in tolerant varieties, highlighting the role of PGRs in enhancing membrane stability under stress.

Free Proline

The free proline content (mg/g fresh weight, FW) was measured in various groundnut varieties subjected to different PEG concentrations (10% to 25%) and grown in petri dishes. The proline levels at 15 days after sowing (DAS) are presented in Table 9.

Among the varieties tested, GJG-31 (V8) exhibited the highest mean free proline content of 0.281 mg/g FW, while the lowest value of 0.203 mg/g FW was recorded in GG-7 (V3). The treatment effect was also significant, with free proline content varying between 0.118 and 0.434 mg/g FW. The control treatment (T0), irrigated with tap water, showed the lowest mean free proline content (0.073 mg/g FW). Free proline content increased progressively with increasing PEG concentrations, indicating a response to drought stress.

The interaction between variety and treatment was significant. The lowest free proline content (0.046 mg/g FW) was observed in GG-7 under control conditions (V3T0), whereas the highest content (0.601 mg/g FW) was recorded in GJG-31 at 25% PEG concentration (V8T4).

Free proline accumulation is widely recognized as a biochemical marker of drought tolerance in plants. Proline acts as an osmoprotectant, helping to maintain cellular osmotic balance during water deficit conditions. In this study, all varieties showed increased proline content with rising drought stress levels, with GJG-31 exhibiting the most pronounced accumulation.

These results corroborate previous findings: Vaidya *et al.* (2015) reported that drought stress impairs crop growth by disrupting physiological and biochemical processes, but genotypes accumulating higher proline showed better drought tolerance through improved water use efficiency. Razi *et al.* (2021) found proline levels increased substantially under drought in okra genotypes, with the highest accumulation in NS7774. Asati *et al.* (2024) observed a significant rise in proline across 78 chickpea genotypes under drought, with tolerant genotypes showing higher fold increases than sensitive ones. Wang *et al.* (2024) reported elevated proline levels under drought in soybean leaves, correlated with increased water use efficiency. Qi *et al.* (2023) demonstrated that proline content in passion fruit seedlings peaked at day 9, with the highest levels under 20% PEG treatment.

Overall, the increase in free proline content with PEG-induced drought stress in groundnut seedlings reflects an adaptive physiological mechanism to mitigate drought effects and maintain cellular homeostasis.

Glycine Betaine

Glycine betaine content (mg/g fresh weight, FW) was measured across different groundnut varieties subjected to various PEG concentrations (10% to 25%) and cultivated in petri dishes. The glycine betaine levels recorded at 15 days after sowing (DAS) are presented in Table 10.

Among the varieties studied, GJG-31 (V8) exhibited the highest mean glycine betaine content of 0.247 mg/g FW, while the lowest value of 0.135 mg/g FW was recorded in GG-7 (V3). Treatment effects were significant, with glycine betaine content ranging from 0.147 to 0.257 mg/g FW. Glycine betaine levels increased significantly with rising PEG-induced drought stress, including the control treatment.

The interaction between variety and treatment was also significant. The lowest glycine betaine content (0.104 mg/g FW) was observed in GG-7 under control conditions (V3T0), while the highest accumulation (0.373 mg/g FW) was recorded in GJG-31 at 25% PEG concentration (V8T4).

Glycine betaine plays a vital role in plant drought tolerance by acting as an osmoprotectant that stabilizes cellular structures and protects photosynthetic machinery under water-deficient conditions. Its accumulation helps maintain cellular osmotic balance, enhances antioxidant defense mechanisms, and protects membranes from oxidative damage (Ishfaq *et al.*, 2024).

Under drought stress, glycine betaine synthesis is upregulated as an essential physiological response, aiding plants in withstanding water deficits by stabilizing membranes, proteins, and enzymes, and reducing oxidative stress (Niu *et al.*, 2021). This accumulation is associated with enhanced resistance to multiple abiotic stresses.

Supporting studies include: Razi *et al.* (2021) found that drought-tolerant okra genotypes accumulated higher glycine betaine levels than sensitive ones, which contributed to maintaining water potential and cellular stability. Huang *et al.* (2022) reported that glycine betaine accumulation in *Artemisia* *selengensis* under drought improved photosynthetic efficiency and reduced lipid peroxidation, enhancing stress adaptation. Ishfaq *et al*. (2024) documented increased glycine betaine content in wheat under drought, with Anaaj-2017 showing the highest accumulation (16% more than other varieties), while Punjab-2011 showed the lowest. Maleki *et al*. (2024) observed that fenugreek accumulated significantly more glycine betaine under drought stress, improving drought resilience by reducing oxidative damage and maintaining membrane integrity.

Overall, the increase in glycine betaine content in groundnut seedlings subjected to PEG-induced drought stress underscores its critical role in drought tolerance mechanisms by protecting cellular components and maintaining physiological stability.

Lipid Peroxidation

Lipid peroxidation levels (nmol/g fresh weight, FW) were assessed in various groundnut seedling varieties exposed to different PEG concentrations (10% to 25%) and cultivated in petri dishes. The lipid peroxidation content of groundnut leaves at 15 days after sowing (DAS) is detailed in Table 11.

Among the varieties studied, GG-7 (V3) recorded the highest mean lipid peroxidation value of 35.30 nmol/g FW, while the lowest level (23.27 nmol/g FW) was observed in GJG-31 (V8). Treatment effects were significant, with lipid peroxidation varying from 24.60 to 39.28 nmol/g FW depending on PEG concentration. The control treatment (T0), irrigated with distilled water, showed the lowest lipid peroxidation value (24.6 nmol/g FW). As PEG-induced drought stress intensified, lipid peroxidation levels increased relative to the control.

The interaction between variety and treatment was significant. The lowest lipid peroxidation (20.24 nmol/g FW) was found in GJG-31 under control conditions (V8T0), whereas the highest (44.62 nmol/g FW) occurred in GG-2 (V2) at 25% PEG treatment (V2T4).

Lipid peroxidation is a critical biochemical marker of oxidative stress in plants under drought conditions. Reactive oxygen species (ROS) such as superoxide radicals (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radicals (OH^-) accumulate under drought, causing oxidative damage to cellular membranes by attacking polyunsaturated fatty acids (PUFAs). This process produces malondialdehyde (MDA), a widely accepted indicator of membrane lipid peroxidation and cellular damage (Razi *et al*., 2021; Ishfaq *et al*., 2024).

Supporting evidence from recent studies highlights this mechanism: Razi *et al*. (2021) reported increased lipid peroxidation in drought-stressed okra due to ROS-induced chloroplast damage. Asati *et al*. (2024) observed significant increases in MDA content across 78 chickpea varieties under drought, with the highest MDA levels found in drought-sensitive genotypes. Ishfaq *et al*. (2024) documented a 722% increase in MDA accumulation in drought-stressed wheat, severely affecting membrane integrity. Maleki *et al*. (2024) noted elevated MDA levels in fenugreek under drought stress, indicating substantial oxidative damage.

Drought-induced lipid peroxidation disrupts membrane fluidity, impairs ion transport, and accelerates cellular senescence, collectively hindering plant growth and yield. Plants with robust antioxidant defense systems such as enhanced activities of superoxide dismutase (SOD), catalase (CAT), and peroxidases (POD) show reduced lipid peroxidation and improved drought tolerance (Huang *et al*., 2022).

Correlation Matrix

Table 12 shows the correlation coefficients among physiological and biochemical parameters. Drought Tolerance Index (DTI) was strongly positively correlated with Relative Water Content (RWC, 0.8857), Membrane Stability Index (MSI, 0.8380), and Germination% (0.7802). Germination% also correlated positively with MSI (0.8196) and DTI (0.7802). Moisture% had a strong correlation with MSI (0.9196) and a moderate correlation with DTI (0.7680). RWC correlated strongly with DTI (0.8857), Chlorophyll-b (0.8072), and Total Chlorophyll (0.7984). All chlorophyll components (Chlorophyll-a, Chlorophyll-b, Total Chlorophyll) were highly inter-correlated (e.g., Chlorophyll-a and Chlorophyll-b: 0.9969). MSI showed strong positive correlations with Moisture% (0.9196), Germination% (0.8196), and DTI (0.8380). Free Proline correlated negatively with Lipid Peroxidation (−0.8559) and positively with Chlorophyll-a (0.7683) and Total Chlorophyll (0.7616). Glycine Betaine correlated positively with Chlorophyll-b (0.8168) and Total Chlorophyll (0.8017). Lipid Peroxidation showed consistent negative correlations with all parameters, especially Free Proline (−0.8559), RWC (−0.7173), and Total Chlorophyll (−0.7118).

Conclusion

The present study demonstrated that drought stress induced by increasing concentrations of PEG-6000 significantly affected the physiological and biochemical traits of groundnut seedlings. Stress led to a marked decline in germination percentage, relative water content (RWC), membrane stability index (MSI), chlorophyll content, and seedling growth, while promoting the accumulation of stress-responsive osmolytes such as proline and glycine betaine, as well as an increase in lipid peroxidation an indicator of oxidative damage. Among the ten groundnut varieties tested, GJG-31 consistently exhibited the highest tolerance to drought stress, as indicated by its superior performance in key traits such as RWC, MSI, chlorophyll content, DTI, and osmolyte accumulation. Conversely, GG-7 was the most susceptible variety across multiple stress indicators.

Correlation analysis revealed that drought tolerance index (DTI) was strongly and positively associated with RWC, MSI, and germination percentage, highlighting these as reliable markers for drought tolerance. Lipid peroxidation, a marker of membrane damage, showed significant negative correlations with proline, RWC, and chlorophyll content, reinforcing the importance of antioxidant and osmoprotective mechanisms in drought resilience. Overall, this study emphasizes the utility of integrating physiological and biochemical markers, alongside correlation analysis, for identifying drought-tolerant genotypes in groundnut breeding programs. The variety GJG-31 emerges as a promising candidate for cultivation under water-limited conditions.

Table 1 : Effect of drought stress on germination (%) of groundnut

Varieties (V)	Drought treatments (T)					
	T0	**T1**	**T2**	**T3**	**T4**	**Mean (V)**
V_1	100.00	96.00	82.00	74.67	69.33	84.40
V_2	100.00	95.33	90.67	84.67	77.33	89.60
V_3	99.33	89.33	80.67	73.33	60.67	80.66
V_4	100.00	94.67	80.00	76.67	70.67	84.40
V_5	100.00	95.33	84.67	76.67	68.67	85.06
V_6	100.00	93.33	87.33	69.33	66.67	83.33
V_7	100.00	94.00	81.33	75.33	69.33	84.00
V_8	100.00	97.33	90.00	84.00	80.67	90.40
V_9	100.00	92.67	82.67	80.67	72.00	85.60
V_{10}	100.00	91.33	82.67	80.00	73.33	85.46
Mean (T)	99.93	93.93	84.20	77.53	70.87	

	S.Em.±	**C.D. at 5%**	**C.V.%**	
V	0.273	0.768		1.24
T	0.193	0.543		
VXT	0.611	1.717		

Whereas, V1:GG-6, **V2**: GG-2, **V3**: GG-7, **V4**:GJG-9, **V5**: GG-11, **V6**: GG-21, **V7**: GJG-22, **V8**: GJG-31, **V9**: GJG-32 and **V10**: GJG-33 and **T0**: Control, **T1**:10% PEG, **T2**: 15% PEG **T3**: 20% PEG and **T4**: 25% PEG

Table 2 : Effect of drought stress on relative water content (RWC) in groundnut seedlings at 15 DAS

Varieties (V)	Drought treatments (T)					
	T0	**T1**	**T2**	**T3**	**T4**	**Mean (V)**
V_1	88.39	86.33	84.50	81.90	78.17	83.85
V_2	88.55	85.36	83.19	81.34	80.14	83.71
V_3	84.30	80.68	78.88	75.41	72.35	78.32
V_4	86.82	85.68	84.70	81.17	77.79	83.23
V_5	85.85	83.44	79.69	75.76	71.90	79.32
V_6	85.74	84.14	80.18	74.96	72.87	79.57
V_7	90.51	89.24	83.91	80.10	76.81	84.11
V_8	96.69	93.71	91.43	87.10	83.70	90.52
V_9	94.32	91.43	86.60	80.40	79.10	86.37
V_{10}	92.41	90.34	84.91	82.40	79.80	85.97
Mean (T)	89.36	87.04	83.80	80.06	77.27	

	S.Em.±	**C.D.at5%**	**C.V.%**	
V	0.272	0.765		1.26
T	0.193	0.541		
VXT	0.609	1.711		

Whereas, V1:GG-6, **V2**: GG-2, **V3**: GG-7, **V4**:GJG-9, **V5**: GG-11, **V6**: GG-21, **V7**: GJG-22, **V8**: GJG-31, **V9**: GJG-32 and **V10**: GJG-33 and **T0**: Control, **T1**:10% PEG, **T2**: 15% PEG **T3**: 20% PEG and **T4**: 25% PEG

Table 3 : Effect of drought stress on drought tolerance index (DTI) in groundnut at 15 DAS

Varieties (V)	Drought treatments (T)					
	T0	T1	T2	T3	T4	Mean (V)
V_1	98.00	75.24	65.87	53.34	48.30	68.15
V_2	98.33	74.20	66.20	61.44	53.04	70.64
V_3	97.00	43.40	40.97	39.24	36.84	51.49
V_4	98.67	76.24	67.17	55.17	47.84	69.01
V_5	98.00	75.57	66.07	52.50	49.57	68.34
V_6	98.33	53.60	50.27	47.74	41.07	58.20
V_7	98.00	74.10	66.60	64.47	51.34	70.90
V_8	99.00	88.14	79.07	70.77	65.67	80.53
V_9	98.00	78.34	75.24	62.24	53.64	73.49
V_{10}	98.00	83.97	77.20	66.24	57.94	76.67
Mean (T)	98.13	72.28	65.47	57.31	50.52	

	S.Em.±	C.D.at5%	C.V.%	1.92
V	0.074	0.209		
T	0.053	0.148		
VXT	0.166	0.466		

Whereas, V1:GG-6, **V2**: GG-2, **V3**: GG-7, **V4**:GJG-9, **V5**: GG-11, **V6**: GG-21, **V7**: GJG-22, **V8**: GJG-31, **V9**: GJG-32 and **V10**: GJG-33 and **T0**: Control, **T1**:10% PEG, **T2**: 15% PEG **T3**: 20% PEG and **T4**: 25% PEG

Table 4 : Effect of drought stress on moisture (%) in groundnut seedlings at 15 DAS

Varieties (V)	Drought treatments (T)					
	T0	T1	T2	T3	T4	Mean (V)
V_1	88.58	81.21	74.00	67.95	62.05	74.76
V_2	90.96	85.92	74.84	67.27	62.38	76.28
V_3	73.77	49.70	46.55	40.26	37.35	49.53
V_4	89.17	81.87	75.97	66.58	60.96	74.91
V_5	86.60	79.96	73.46	67.96	62.77	74.15
V_6	89.05	82.60	74.05	66.82	62.16	74.94
V_7	88.19	80.94	73.41	66.83	60.94	74.07
V_8	97.89	84.07	76.25	68.40	63.14	77.95
V_9	92.19	82.94	75.41	67.96	62.84	76.27
V_{10}	89.86	82.90	75.26	65.13	61.94	75.02
Mean (T)	88.63	79.21	71.92	64.52	59.66	

	S.Em.±	C.D.at5%	C.V.%	1.57
V	0.295	0.827		
T	0.208	0.585		
VXT	0.659	1.850		

Whereas, V1:GG-6, **V2**: GG-2, **V3**: GG-7, **V4**:GJG-9, **V5**: GG-11, **V6**: GG-21, **V7**: GJG-22, **V8**: GJG-31, **V9**: GJG-32 and **V10**: GJG-33 and **T0**: Control, **T1**:10% PEG, **T2**: 15% PEG **T3**: 20% PEG and **T4**: 25% PEG

Table 5 : Effect of drought stress on chlorophyll A content (mg/g FW) in groundnut seedlings at 15 DAS

Varieties (V)	Drought treatments (T)					
	T0	T1	T2	T3	T4	Mean (V)
V_1	0.069	0.066	0.059	0.056	0.041	0.058
V_2	0.072	0.070	0.060	0.055	0.044	0.060
V_3	0.065	0.064	0.058	0.050	0.037	0.055
V_4	0.085	0.083	0.077	0.066	0.058	0.074
V_5	0.076	0.070	0.066	0.060	0.056	0.066
V_6	0.075	0.071	0.065	0.061	0.056	0.066
V_7	0.083	0.079	0.072	0.070	0.062	0.073
V_8	0.096	0.096	0.090	0.083	0.070	0.087
V_9	0.084	0.084	0.082	0.072	0.060	0.076
V_{10}	0.087	0.085	0.074	0.070	0.063	0.076
Mean (T)	0.080	0.077	0.071	0.065	0.055	

	S.Em.±	C.D.at5%	C.V.%	4.4
V	0.01	0.002		
T	0.01	0.002		
VXT	0.002	0.005		

Whereas, V1:GG-6, **V2**: GG-2, **V3**: GG-7, **V4**:GJG-9, **V5**: GG-11, **V6**: GG-21, **V7**: GJG-22, **V8**: GJG-31, **V9**: GJG-32 and **V10**: GJG-33 and **T0**: Control, **T1**:10% PEG, **T2**: 15% PEG **T3**: 20% PEG and **T4**: 25% PEG

Table 6 : Effect of drought stress on chlorophyll B content (mg/g FW) in groundnut seedlings at 15 DAS

Varieties (V)	Drought treatments (T)					
	T0	T1	T2	T3	T4	Mean (V)
V_1	0.062	0.058	0.052	0.048	0.031	0.050
V_2	0.064	0.062	0.052	0.049	0.037	0.053
V_3	0.057	0.056	0.051	0.042	0.029	0.047
V_4	0.075	0.074	0.067	0.057	0.048	0.064
V_5	0.067	0.061	0.057	0.051	0.047	0.057
V_6	0.067	0.062	0.057	0.053	0.049	0.058
V_7	0.076	0.072	0.064	0.062	0.054	0.066
V_8	0.088	0.088	0.082	0.075	0.063	0.079
V_9	0.077	0.076	0.074	0.064	0.052	0.069
V_{10}	0.078	0.075	0.064	0.061	0.054	0.066
Mean (T)	0.071	0.069	0.062	0.057	0.047	

	S.Em. ±	C.D.at5%	C.V.%	4.12
V	0.001	0.002		
T	0.001	0.001		
VXT	0.002	0.004		

Whereas, V1:GG-6, **V2**: GG-2, **V3**: GG-7, **V4**:GJG-9, **V5**: GG-11, **V6**: GG-21, **V7**: GJG-22, **V8**: GJG-31, **V9**: GJG-32 and **V10**: GJG-33 and **T0**: Control, **T1**:10% PEG, **T2**: 15% PEG **T3**: 20% PEG and **T4**: 25% PEG

Table 7 : Effect of drought stress on total chlorophyll content (mg/g FW) in groundnut seedlings at 15 DAS

Varieties (V)	Drought treatments (T)					
	T0	T1	T2	T3	T4	Mean (V)
V_1	0.132	0.125	0.112	0.105	0.073	0.109
V_2	0.137	0.133	0.113	0.105	0.082	0.114
V_3	0.123	0.121	0.110	0.093	0.067	0.103
V_4	0.161	0.158	0.145	0.124	0.107	0.139
V_5	0.144	0.132	0.124	0.112	0.104	0.123
V_6	0.143	0.134	0.123	0.115	0.106	0.124
V_7	0.160	0.152	0.137	0.133	0.117	0.140
V_8	0.185	0.185	0.173	0.159	0.134	0.167
V_9	0.162	0.161	0.157	0.137	0.113	0.146
V_{10}	0.166	0.161	0.139	0.132	0.118	0.143
Mean (T)	0.151	0.146	0.133	0.121	0.102	

	S.Em.±	C.D.at5%	C.V.%	4.22
V	0.001	0.004		
T	0.001	0.003		
VXT	0.003	0.009		

Whereas, V1:GG-6, **V2**: GG-2, **V3**: GG-7, **V4**:GJG-9, **V5**: GG-11, **V6**: GG-21, **V7**: GJG-22, **V8**: GJG-31, **V9**: GJG-32 and **V10**: GJG-33 and **T0**: Control, **T1**:10% PEG, **T2**: 15% PEG **T3**: 20% PEG and **T4**: 25% PEG

Table 8 : Effect of drought stress on membrane stability index (MSI) in groundnut seedlings at 15 DAS

Varieties (V)	Drought treatments (T)					
	T0	T1	T2	T3	T4	Mean (V)
V_1	75.14	67.77	60.56	54.51	48.61	61.32
V_2	77.52	72.48	61.40	53.83	48.94	62.84
V_3	54.15	50.06	46.13	41.13	38.04	45.90
V_4	75.73	68.43	62.53	53.14	47.52	61.47
V_5	73.16	66.52	60.02	54.52	49.33	60.71
V_6	75.61	69.16	60.61	53.38	48.72	61.50
V_7	74.75	67.50	59.97	53.39	47.50	60.62
V_8	86.07	79.84	73.08	65.93	50.96	71.18
V_9	78.75	69.50	61.97	54.52	49.40	62.83
V_{10}	76.42	69.46	61.82	51.69	48.50	61.58
Mean (T)	74.73	68.07	60.81	53.61	47.75	

	S.Em.±	C.D.at5%	C.V.%	1.87
V	0.295	0.829		
T	0.209	0.586		
VXT	0.660	1.853		

Whereas, V1:GG-6, **V2**: GG-2, **V3**: GG-7, **V4**:GJG-9, **V5**: GG-11, **V6**: GG-21, **V7**: GJG-22, **V8**: GJG-31, **V9**: GJG-32 and **V10**: GJG-33 and **T0**: Control, **T1**:10% PEG, **T2**: 15% PEG **T3**: 20% PEG and **T4**: 25% PEG

Table 9 : Effect of drought stress on free proline (mg/g FW) in groundnut seedlings at 15 DAS

Varieties (V)	Drought treatments (T)					
	T0	T1	T2	T3	T4	Mean (V)
V_1	0.055	0.111	0.245	0.304	0.375	0.218
V_2	0.071	0.119	0.247	0.303	0.367	0.221
V_3	0.046	0.117	0.220	0.272	0.361	0.203
V_4	0.062	0.120	0.284	0.316	0.446	0.246
V_5	0.057	0.107	0.249	0.312	0.492	0.243
V_6	0.087	0.128	0.239	0.297	0.372	0.225
V_7	0.089	0.114	0.210	0.229	0.449	0.218
V_8	0.089	0.124	0.283	0.308	0.601	0.281
V_9	0.086	0.121	0.193	0.273	0.440	0.223
V_{10}	0.088	0.121	0.222	0.300	0.443	0.235
Mean (T)	0.073	0.118	0.239	0.291	0.434	

	S.Em.±	C.D.at5%	C.V.%	
V	0.001	0.003	C.V.%	2.06
T	0.001	0.003		
VXT	0.003	0.008		

Whereas, V1:GG-6, **V2**: GG-2, **V3**: GG-7, **V4**:GJG-9, **V5**: GG-11, **V6**: GG-21, **V7**: GJG-22, **V8**: GJG-31, **V9**: GJG-32 and **V10**: GJG-33 and **T0**: Control, **T1**:10% PEG, **T2**: 15% PEG **T3**: 20% PEG and **T4**: 25% PEG

Table 10 : Effect of drought stress on glycine betaine (mg/g FW) in groundnut seedling at 15 DAS

Varieties (V)	Drought treatments (T)					
	T0	T1	T2	T3	T4	Mean (V)
V_1	0.125	0.144	0.155	0.185	0.217	0.165
V_2	0.117	0.133	0.175	0.182	0.238	0.169
V_3	0.104	0.112	0.126	0.153	0.180	0.135
V_4	0.115	0.126	0.133	0.185	0.210	0.154
V_5	0.118	0.143	0.156	0.189	0.248	0.171
V_6	0.126	0.152	0.196	0.240	0.268	0.196
V_7	0.129	0.152	0.179	0.194	0.251	0.181
V_8	0.156	0.188	0.225	0.291	0.373	0.247
V_9	0.144	0.166	0.215	0.270	0.320	0.223
V_{10}	0.127	0.153	0.175	0.209	0.258	0.185
Mean (T)	0.126	0.147	0.174	0.210	0.257	

	S.Em.±	C.D.at 5%	C.V.%	
V	0.001	0.004	C.V.%	2.68
T	0.001	0.003		
VXT	0.003	0.008		

Whereas, V1:GG-6, **V2**: GG-2, **V3**: GG-7, **V4**:GJG-9, **V5**: GG-11, **V6**: GG-21, **V7**: GJG-22, **V8**: GJG-31, **V9**: GJG-32 and **V10**: GJG-33 and **T0**: Control, **T1**:10% PEG, **T2**: 15% PEG **T3**: 20% PEG and **T4**: 25% PEG

Table 11 : Effect of drought stress on lipid peroxidation (nmol/g FW) in groundnut seedlings at 15 DAS

Varieties (V)	Drought treatments (T)					
	T0	T1	T2	T3	T4	Mean (V)
V_1	23.86	27.53	33.90	36.600	39.78	32.33
V_2	25.79	26.31	33.08	39.86	44.62	33.93
V_3	27.32	30.78	37.19	39.86	41.36	35.30
V_4	23.36	28.11	33.96	36.82	40.49	32.55
V_5	26.94	29.93	33.86	36.08	39.39	33.24
V_6	24.44	27.36	33.58	37.53	40.52	32.69
V_7	23.90	28.36	34.43	37.76	40.32	32.95
V_8	20.24	21.18	23.20	24.36	27.36	23.27
V_9	26.81	29.53	37.36	39.40	41.32	34.88
V_{10}	23.36	24.86	29.11	32.15	37.70	29.44
Mean (T)	24.60	27.39	32.96	36.04	39.28	

	S.Em.±	C.D.at5%	C.V.%	
V	0.263	0.740	C.V.%	3.18
T	0.186	0.523		
VXT	0.589	1.655		

Whereas, V1:GG-6, **V2**: GG-2, **V3**: GG-7, **V4**:GJG-9, **V5**: GG-11, **V6**: GG-21, **V7**: GJG-22, **V8**: GJG-31, **V9**: GJG-32 and **V10**: GJG-33 and **T0**: Control, **T1**:10% PEG, **T2**: 15% PEG **T3**: 20% PEG and **T4**: 25% PEG

Table 12 : Correlation coefficients between various physiological and biochemical parameters

	DTI	Germination %	Moisture %	RWC	Chloro-phyll-a	Chloro-phyll-b	Total Chlorophyll	MSI	Free Proline	Glycine Betaine	Lipid Peroxidation
DTI	1.0000										
Germination%	0.7802	1.0000									
Moisture%	0.7680	0.6631	1.0000								
RWC	0.8857	0.7326	0.5794	1.0000							
Chlorophyll-a	0.7743	0.5126	0.5645	0.7886	1.0000						
Chlorophyll-b	0.7728	0.5343	0.5611	0.8072	0.9969	1.0000					
Total Chlorophyll	0.7741	0.5238	0.5633	0.7984	0.9992	0.9992	1.0000				
MSI	0.8380	0.8196	0.9196	0.7594	0.7216	0.7301	0.7264	1.0000			
Free Proline	0.6631	0.6595	0.5257	0.6042	0.7683	0.7536	0.7616	0.7535	1.0000		
Glycine Betaine	0.6506	0.6199	0.5970	0.7412	0.7856	0.8168	0.8017	0.7807	0.6150	1.0000	
Lipid Peroxidation	-0.6371	-0.6123	-0.4045	-0.7173	-0.7106	-0.7120	-0.7118	-0.6829	-0.8559	-0.6425	1.0000

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