

Abiotic Stress and Seed Germination: Insights from Drought Adapted Plants of Arid Indian Regions

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Abstract: This study investigates the impact of abiotic stress, particularly drought, on seed germination in drought-adapted plant species native to the arid regions of India, such as Rajasthan and Gujarat. The research aims to understand how water-deficit conditions affect germination dynamics and early seedling development. Laboratory experiments simulated drought conditions using varying concentrations of polyethylene glycol (PEG-6000) to induce osmotic stress. Selected plant species were evaluated for germination percentage, mean germination time, seedling vigor, and biochemical markers including proline accumulation and antioxidant activity. Results revealed significant interspecies variation in drought tolerance, with certain native species demonstrating robust germination performance even under high stress. The findings highlight the adaptive mechanisms of these species and underscore their potential role in sustainable agriculture and ecosystem restoration in water-scarce environments. This research contributes to the development of drought-resilient crops and supports strategic planning for agriculture in regions increasingly affected by climate variability.

Keywords: PEG-6000, Seed Germination, PEG, Drought Simulation, Dryland Agriculture.

I. Introduction

Abiotic stress encompasses the harmful effects of non-living environmental factors on living organisms, especially plants. Key forms of such stress include drought, salinity, extreme temperatures, and nutrient imbalances. Among these, drought stands out as a major limitation to plant growth, productivity, and survival, particularly in arid and semi-arid ecosystems. It restricts water absorption, disrupts cellular functions, and interferes with vital physiological activities, including seed germination and early seedling development. Seed germination marks a critical stage in a plant's life cycle, laying the foundation for growth and crop establishment. Under drought conditions, this process can be delayed or inhibited due to insufficient water, which impedes enzymatic activation and the mobilization of stored nutrients. Understanding seed responses to moisture deficit is therefore crucial for identifying and developing drought-resilient plant varieties.

India's arid and semi-arid regions—spanning Rajasthan, Gujarat, Maharashtra, and Andhra Pradesh—are marked by low, erratic rainfall, high evapotranspiration, and nutrient-poor soils. Despite these challenges, many native species have evolved to thrive under water-limited conditions through morphological, physiological, and biochemical adaptations. These include deep rooting systems, thickened cuticles, osmotic regulation, and the accumulation of protective solutes like proline. In the face of climate change and the increasing incidence of drought, research on drought-tolerant plants has gained urgency. Utilizing naturally adapted species can contribute to more resilient agriculture and support ecological restoration efforts in degraded and desertified regions.

The present study aims to investigate the impact of drought-induced abiotic stress on the germination of selected native drought-tolerant species from India's arid zones. By simulating drought stress in a controlled laboratory setting using polyethylene glycol (PEG), the study evaluates germination traits, tolerance levels, and biochemical responses. The outcomes are expected to inform breeding programs and sustainable land management strategies suitable for water-scarce environments.

II. Literature Review

Seed germination under abiotic stress, particularly drought, has been widely studied due to its critical role in plant establishment and crop productivity. Drought affects water uptake, enzyme activation, and energy metabolism, thereby delaying or inhibiting germination. Several studies have used polyethylene glycol (PEG) to simulate drought conditions, enabling controlled investigation of osmotic stress on seeds (Michel & Kaufmann, 1973). Research on arid-zone species such as *Prosopis cineraria* and *Cenchrus ciliaris* shows inherent adaptability to low-moisture environments due to physiological traits like hard seed coats and rapid metabolic response (Gupta & Tyagi, 1992; Chauhan et al., 2009). However, most studies focus either on cultivated crops or do not include a comparative biochemical analysis. This study addresses these gaps by evaluating both germination and stress-response markers (e.g., proline, antioxidants) in native drought-tolerant species. Such insights are essential for identifying resilient plant materials for reforestation, pasture development, and sustainable agriculture in arid Indian regions.

III. Materials and Methods

Plant Selection

The selection of plant species for this study was based on their known drought tolerance and prevalence in the arid regions of India, particularly the Thar Desert in Rajasthan and parts of Gujarat. These species have been historically recognized for their ecological resilience and utility in dryland agriculture and ecological restoration.

The following drought-tolerant species were selected:

Prosopis cineraria (Khejri): A leguminous tree native to the Thar Desert, valued for its drought resistance, nitrogen fixation ability, and fodder quality.

Cenchrus ciliaris (Buffel Grass): A perennial grass commonly used for pasture, known for its adaptability to arid conditions and rapid germination.

Tephrosia purpurea (Wild Indigo): A hardy legume with known medicinal and soil-enriching properties, capable of surviving in marginal, dryland soils.

Cassia angustifolia (Senna): A medicinal shrub that thrives in sandy, dry soils and shows potential for desert agriculture.

Zizyphus nummularia (Jharber): A shrub used in agroforestry systems with excellent drought endurance and multiple uses in traditional medicine and livestock feed.

Certified seeds were collected from the Central Arid Zone Research Institute (CAZRI), Jodhpur, and local agricultural extension centers. Seeds were cleaned and stored in airtight containers under cool, dry conditions until use.

Experimental Design

Seed Preparation and Pre-treatment

Prior to the experiment, seeds of all species underwent surface sterilization using 0.1% mercuric chloride solution for 2 minutes, followed by thorough washing with distilled water. This was done to prevent microbial contamination during germination trials.

To overcome seed dormancy (if present), species-specific pre-treatments were applied:

- *Prosopis cineraria* and *Zizyphus nummularia* seeds were scarified mechanically.
- *Tephrosia purpurea* and *Cassia angustifolia* underwent warm water treatment (soaked at 60°C for 10 minutes).
- *Cenchrus ciliaris* seeds were used without dormancy treatment due to their naturally high germination rates.

Drought Simulation

Polyethylene glycol (PEG-6000) was used to simulate drought stress by inducing osmotic potential in the germination medium. PEG is an inert, high-molecular-weight polymer that reduces water availability without being absorbed by plant tissues.

Four levels of PEG treatment were prepared based on water potential levels:

- **Control (0% PEG)** – No osmotic stress
- **Low stress (-0.3 MPa):** 10% PEG
- **Moderate stress (-0.6 MPa):** 15% PEG
- **High stress (-0.9 MPa):** 20% PEG

The water potentials were calculated using the Michel and Kaufmann equation for PEG solutions.

Germination Setup

The germination experiment was conducted in a controlled growth chamber. Sterile Petri dishes (9 cm diameter) were lined with Whatman No. 1 filter paper, moistened with 5 ml of the respective PEG solution, and sown with 25 uniform seeds of each species per dish. Each treatment was replicated three times ($n = 3$).

The Petri dishes were incubated under the following standardized conditions:

- **Temperature:** $25 \pm 2^\circ\text{C}$
- **Photoperiod:** 12 hours light / 12 hours dark
- **Relative Humidity:** 65–70%
- **Monitoring Period:** 10 days

PEG solutions were replenished every 48 hours to maintain consistent osmotic pressure.

Data Collection

Germination Parameters

Germination was monitored daily, and seeds were considered germinated when the radicle reached at least 2 mm in length. The following parameters were recorded:

Germination Percentage (GP):

$$GP = \left(\frac{\text{Number of seeds germinated}}{\text{Total seeds sown}} \right) \times 100$$

Mean Germination Time (MGT):

$$MGT = \frac{\sum (n_i \times t_i)}{\sum n_i}$$
 where n_i is the number of seeds germinated on day t_i .

Seedling Vigor Index (SVI):

$$SVI = \text{Germination \%} \times \text{Mean seedling length (cm)}$$

Measurements of root and shoot lengths were taken on the 10th day using a digital vernier caliper.

Biochemical Parameters

To understand the physiological responses to drought stress, two key biochemical parameters were measured from 10-day-old seedlings:

Proline Content:

Proline accumulation, a stress-induced osmoprotectant, was estimated using the Bates et al. (1973) method. Fresh seedling tissue (0.5 g) was homogenized in 3% sulfosalicylic acid and reacted with acid ninhydrin and glacial acetic acid. The absorbance was read at 520 nm using a UV-Vis spectrophotometer.

Antioxidant Activity:

Antioxidant activity was assessed via the DPPH radical scavenging assay. Methanolic extracts of seedlings were mixed with 0.1 mM DPPH solution, and the reduction in absorbance at 517 nm was measured after 30 minutes of incubation in the dark. Results were expressed as percentage inhibition.

Statistical Analysis

Data were compiled and analyzed using SPSS version 25 and Microsoft Excel. All experiments were performed in triplicates, and results were expressed as mean $\pm$ standard error (SE).

A one-way Analysis of Variance (ANOVA) was conducted to determine the statistical significance of differences in germination and biochemical parameters across PEG treatments and plant species. Post hoc Tukey's HSD test was applied for pairwise comparison of means.

The level of significance was set at $p < 0.05$. Graphs and data visualizations (bar charts and line graphs) were generated using GraphPad Prism software.

This methodology was designed to simulate field-like drought conditions in a controlled lab setting while enabling precise measurement of both physiological and biochemical germination responses. The use of both native species and multiple PEG concentrations provides a robust platform to evaluate stress tolerance and select potential candidates for future dryland agriculture and ecological restoration initiatives.

IV. Results

The results of the study provide a comprehensive comparison of seed germination performance and biochemical responses of selected drought-tolerant plant species under varying levels of PEG-induced drought stress. The findings are presented in terms of germination parameters (germination percentage, mean germination time, and seedling vigor index) and biochemical markers (proline content and antioxidant activity).

Germination Performance under Drought Stress

The effect of increasing PEG concentrations on germination percentage is summarized in **Table 1** and **Figure 1**.

Table 1: Germination Percentage (%) under Different PEG Concentrations

Species	Control (0%)	10% PEG	15% PEG	20% PEG
Species	Control (0%)	10% PEG	15% PEG	20% PEG
<i>Prosopis cineraria</i>	92 $\pm$ 2.1	85 $\pm$ 2.5	73 $\pm$ 3.0	58 $\pm$ 3.2
<i>Cenchrus ciliaris</i>	96 $\pm$ 1.5	89 $\pm$ 1.8	81 $\pm$ 2.1	65 $\pm$ 2.7
<i>Tephrosia purpurea</i>	88 $\pm$ 2.4	78 $\pm$ 2.6	66 $\pm$ 2.9	51 $\pm$ 3.1
<i>Cassia angustifolia</i>	84 $\pm$ 2.0	72 $\pm$ 2.3	60 $\pm$ 2.8	47 $\pm$ 3.0

<i>Zizyphus nummularia</i>	90 ± 1.8	83 ± 2.2	70 ± 2.5	56 ± 2.9
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A consistent decline in germination percentage was observed across all species as PEG concentration increased. *Cenchrus ciliaris* showed the highest germination across all treatments, maintaining 65% even at 20% PEG. In contrast, *Cassia angustifolia* was the most sensitive species, with only 47% germination at the highest stress level.

Mean Germination Time (MGT) increased significantly with rising stress levels, indicating delayed germination under water-deficit conditions. Seedling Vigor Index (SVI) also declined proportionally, with *Prosopis cineraria* and *Cenchrus ciliaris* retaining relatively higher vigor compared to others.

Biochemical Responses to Drought Stress

Biochemical analyses showed notable increases in both proline content and antioxidant activity under PEG-induced drought stress.

Table 2: Proline Content (µmol/g fresh weight) under Different PEG Levels

Species	Control	10% PEG	15% PEG	20% PEG
<i>Prosopis cineraria</i>	2.1	3.8	5.4	6.7
<i>Cenchrus ciliaris</i>	1.9	3.5	5.0	6.2
<i>Tephrosia purpurea</i>	2.3	4.0	5.7	6.9
<i>Cassia angustifolia</i>	1.7	3.2	4.6	5.5

Species	Control	10% PEG	15% PEG	20% PEG
<i>Zizyphus nummularia</i>	2.0	3.6	5.2	6.4

Proline content increased significantly ($p < 0.05$) with higher PEG concentrations. *Tephrosia purpurea* showed the highest proline accumulation at 20% PEG, suggesting strong osmotic adjustment ability.

Antioxidant activity, measured as DPPH radical scavenging percentage, also increased with stress. This indicates an elevated production of reactive oxygen species (ROS) and activation of antioxidant defense systems.

Species-Wise Tolerance Comparison

Overall, *Cenchrus ciliaris* and *Prosopis cineraria* exhibited the best drought tolerance based on higher germination percentages, shorter MGT, higher SVI, and substantial biochemical resilience. *Tephrosia purpurea* showed strong biochemical responses but relatively moderate germination performance. *Cassia angustifolia* consistently performed the lowest across all parameters, indicating lesser drought tolerance.

These results confirm that native arid-zone species possess varying degrees of drought adaptability, with some maintaining physiological function even under high osmotic stress. The germination and biochemical data support their potential application in dryland agriculture and restoration ecology.

V. Discussion

Impact of Drought Stress on Germination: The study demonstrated a clear negative correlation between increasing drought stress (PEG concentration) and seed germination parameters. Reduced water availability under osmotic stress inhibited imbibition, delayed metabolic activation, and slowed down or suppressed radicle emergence. Mean germination time (MGT) increased with stress levels, indicating delayed germination due to lower enzymatic activity and impaired mobilization of food reserves.

Decline in Seedling Vigor: Seedling vigor index (SVI) declined significantly across all species under drought stress. This reduction suggests that even when germination occurred, seedling growth was adversely affected, likely due to disrupted cell division and elongation under low water potential.

Comparative Performance of Species: *Cenchrus ciliaris* showed the highest resilience, maintaining >65% germination at 20% PEG, indicating strong drought adaptability.

Prosopis cineraria also performed well, with relatively high vigor and germination under stress. *Tephrosia purpurea* exhibited moderate germination performance but showed significant biochemical resilience (proline accumulation and antioxidant activity). *Cassia angustifolia* was the most sensitive, showing the steepest decline in all germination parameters. *Zizyphus nummularia* performed intermediately, indicating moderate tolerance.

Biochemical Indicators of Tolerance: Increased proline accumulation under drought stress was observed across all species,

especially in *Tephrosia purpurea*, suggesting active osmotic adjustment. Enhanced antioxidant activity indicated an activated defense mechanism against oxidative damage caused by reactive oxygen species (ROS), which are typically elevated under stress.

Physiological and Genetic Traits Involved: Tolerance may be linked to traits such as seed coat permeability, hormonal regulation (ABA sensitivity), and osmolyte synthesis. Genetic traits controlling early stress perception and efficient ROS-scavenging enzymes (e.g., SOD, CAT) likely contribute to the observed variation. Deep dormancy or hard-seededness might also influence delayed germination under stress conditions.

Implications for Dryland Agriculture: Identifying species with high germination potential under drought stress is crucial for developing resilient cropping systems. These findings support the use of *Cenchrus ciliaris*, *Prosopis cineraria*, and *Tephrosia purpurea* in arid-zone agroforestry, pasture development, and reforestation efforts.

Relevance to Seed Technology: The results can inform seed priming strategies and genetic screening for drought tolerance. Incorporating biochemical screening (e.g., proline, antioxidants) can enhance seed selection protocols for stress-prone environments.

VI. Conclusion

This study evaluated the effects of simulated drought stress on the seed germination and early seedling growth of five drought-adapted species from arid regions of India: *Prosopis cineraria*, *Cenchrus ciliaris*, *Tephrosia purpurea*, *Cassia angustifolia*, and *Zizyphus nummularia*. Using PEG-6000 to induce controlled osmotic stress, we observed marked differences in germination percentage, mean germination time, seedling vigor, and biochemical responses across species and treatment levels.

The key findings highlight that increasing drought stress leads to a significant reduction in germination rate and seedling vigor. However, species differed in their tolerance levels. *Cenchrus ciliaris* and *Prosopis cineraria* consistently outperformed others under all stress levels, maintaining higher germination rates and vigor, even at the highest PEG concentration. *Tephrosia purpurea* exhibited notable physiological responses, such as high proline accumulation and antioxidant activity, suggesting effective biochemical defense mechanisms despite moderate germination performance.

The biochemical assays further confirmed that drought stress triggers an increase in proline content and antioxidant activity, both of which serve as indicators of stress tolerance. These traits can be used to screen and select stress-resilient species or genotypes for targeted ecological and agricultural applications.

Based on these findings, we recommend the inclusion of drought-tolerant native species such as *Cenchrus ciliaris*, *Prosopis cineraria*, and *Tephrosia purpurea* in reforestation programs, pasture rehabilitation, and dryland farming systems in arid and semi-arid zones of India. These species not only ensure better seedling establishment under water-limited conditions but also contribute to ecological stability and soil improvement.

There is significant potential for future research in this domain. Detailed physiological and genetic studies can help uncover the molecular mechanisms behind drought tolerance, enabling the development of improved cultivars through selective breeding or genetic engineering. Additionally, field-level validation of laboratory findings and long-term studies on plant survival and productivity under natural drought conditions will further strengthen the application of such species in climate-resilient agriculture and land restoration initiatives.

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