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Seed Germination of Tropical Trees under Polyethylene Glycol-Induced Osmotic Stress: A Comprehensive Study for Early Screening of Drought Tolerance

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A B S T R A C T

Drought-induced water stress is a major environmental factor affecting seed germination and plant establishment in tropical forest ecosystems. This study examined the effects of polyethylene glycol (PEG)-induced osmotic stress on the germination of seven tropical tree species: Hardwickia binnata, Butea monosperma, Acacia catechu, Acacia nilotica, Holoptelea integrifolia, Diospyros melanoxylon, and Pithecellobium dulce. Seeds were subjected to three osmotic levels: 0 MPa (control), −0.5 MPa (moderate stress), and −1.0 MPa (severe stress). Germination experiments were conducted under controlled laboratory conditions using a factorial completely randomized design.

Results showed that increasing osmotic stress significantly reduced germination in all species. Under control conditions, Hardwickia binnata showed the highest germination (90%), while other species exhibited moderate germination rates. Under moderate stress (−0.5 MPa), germination declined substantially, though Hardwickia binnata and Butea monosperma maintained relatively higher tolerance. Severe stress (−1.0 MPa) almost completely inhibited germination in most species. Statistical analysis confirmed significant effects of species, treatment, and their interaction on germination response.

The findings indicate strong species-specific differences in drought tolerance during the germination stage. Hardwickia binnata and Butea monosperma demonstrated greater resilience to water stress and may be suitable for afforestation and restoration programs in drought-prone regions.

1. INTRODUCTION

Water stress in plants occurs when the demand for water exceeds the available supply, a condition that can be induced by various factors including drought, salinity, and extreme temperatures. Among these, drought represents the most pervasive and economically significant environmental constraint limiting plant growth and productivity across terrestrial ecosystems worldwide. Plants have evolved a remarkable array of biochemical, physiological, and morphological adaptations to cope with water deficit, but the severity and frequency of drought events are increasingly exceeding the adaptive capacity of many species, particularly in regions already characterized by water scarcity. The critical soil water potential required for successful seed germination is not uniform across species but rather represents a characteristic physiological threshold unique to each plant species, reflecting its evolutionary history and ecological niche. When soil moisture falls below this critical threshold, germination is impaired or completely inhibited, with cascading effects on subsequent seedling emergence, stand establishment, and ultimately, ecosystem productivity and composition.

Inadequate soil moisture during the germination phase results in reduced and unsynchronised seedling emergence, leading to poor stand establishment and negative impacts on overall productivity. This is particularly consequential in forestry and ecological restoration contexts, where successful establishment depends on the timely and uniform germination of planted or naturally dispersed seeds. The germination process represents the beginning of the plant life cycle, and successful seedling emergence is fundamental for the establishment and persistence of plant populations. When water stress occurs during this critical phase, it can create demographic bottlenecks that shape the future composition and structure of plant communities. Understanding the germination responses of different species to water deficit is therefore essential for predicting how plant populations and communities will respond to changing environmental conditions, and for designing effective strategies for forest regeneration, afforestation, and conservation.

Drought stress exerts its effects through multiple interconnected mechanisms that collectively impair seed germination and early seedling growth. Reduced water availability limits cell expansion and division, compromises membrane integrity and function, and disrupts the delicate balance of cellular metabolism. One of the most damaging consequences of drought stress is the overproduction of reactive oxygen species, which can cause oxidative damage to proteins, lipids, and nucleic acids, leading to cellular dysfunction and death. Drought stress also promotes leaf senescence and reduces photosynthetic capacity, further compromising the plant's ability to sustain growth and development. In the context of seed germination, water stress primarily acts by limiting water imbibition, the initial physical process by which dry seeds take up water and rehydrate their tissues. Without adequate imbibition, the metabolic processes necessary for germination cannot be initiated, and the mobilization of stored reserves is impaired, preventing radicle emergence and subsequent seedling growth.

The survival and persistence of tree species in tropical ecosystems depend critically on their capacity to tolerate drought, particularly in regions characterized by pronounced dry seasons and increasing aridity under climate change. Tropical forests, despite their image as perpetually wet environments, experience seasonal drought in many regions, and the tree species that inhabit these areas have evolved diverse strategies for coping with periodic water scarcity. However, the unprecedented pace of climate change is pushing many species beyond their adaptive limits, and understanding the mechanisms of drought tolerance during the most vulnerable life stages has become an urgent research priority. The germination phase represents a particularly critical bottleneck, as seeds must successfully imbibe water, activate metabolism, and produce a radicle capable of penetrating dry or drying soil. Species that can maintain reasonable germination under moderate water stress may have a significant advantage in establishing populations in increasingly arid environments.

To study plant responses to water stress under controlled laboratory conditions, researchers have developed methods using osmotic solutions with precisely defined water potentials. These solutions allow for the simulation of drought stress without the confounding effects of soil variability, enabling systematic investigation of the physiological and biochemical mechanisms underlying drought tolerance. Among the various osmotic agents available, polyethylene glycol, particularly the 6000 molecular weight form, has emerged as the preferred compound for simulating drought stress in germination studies. Polyethylene glycol 6000 is widely used because its high molecular weight prevents it from penetrating plant cell walls, thereby reducing external water potential without inducing chemical toxicity or interfering directly with cellular metabolism. By dissolving polyethylene glycol in water at different concentrations, researchers can create solutions with accurately calibrated osmotic potentials, allowing for the establishment of precise and reproducible gradients of water stress.

This technique of polyethylene glycol-induced osmotic stress has proven invaluable for the early identification of drought-tolerant individuals and populations based on germination rates, seedling vigour, and physiological responses under water-limited conditions. The ability to screen large numbers of seeds under controlled conditions provides a cost-effective and time-efficient method for assessing genetic variability in drought tolerance, identifying promising genotypes for breeding programs, and selecting suitable species and provenances for restoration projects in drought-prone areas. Numerous studies have successfully employed this approach to evaluate drought tolerance in a wide range of crop and tree species, demonstrating its utility as a screening tool for climate-resilient germplasm.

Seed germination experiments conducted under polyethylene glycol-induced stress have been instrumental in identifying genotypes and provenances of tree species with enhanced drought tolerance, providing valuable information for forest managers and restoration practitioners. Recent research has documented plant responses to polyethylene glycol-induced drought stress during seed germination and early seedling growth in various tree species, contributing to our understanding of species-specific tolerance mechanisms and thresholds. However, despite the growing body of research on agricultural crops and temperate tree species, studies examining the effects of water stress on seed germination of tropical forest tree species remain surprisingly limited. This research gap is particularly concerning given the high biodiversity and ecological importance of tropical forests, and the urgent need to develop effective strategies for their conservation and restoration under increasing drought frequency and intensity.

Addressing this knowledge gap is essential for developing science-based approaches to forest regeneration, afforestation, and conservation that can succeed in the face of climate change. By understanding how different tropical tree species respond to water stress during germination, we can make informed decisions about which species to prioritize for planting in drought-prone areas, how to design seed-based restoration programs, and where to focus conservation efforts to protect the most vulnerable species. The present study was therefore conducted to evaluate the effects of polyethylene glycol-induced water stress on the seed germination behaviour of seven selected tropical forest tree species, all native to the dry deciduous forests of central India. By analysing germination responses under different osmotic stress levels, this study seeks to provide insights into species-specific drought tolerance during the critical early growth stage, and to generate knowledge that can support climate-resilient forest restoration and management practices.

2. MATERIALS AND METHODS

2.1 Seed Collection and Processing

The seeds used in this experiment were collected from mature, healthy trees growing in the tropical deciduous forests of the Koroon ranges, located within the Prayagraj district of Uttar Pradesh, India. The precise geographical coordinates of the collection site are latitude 24 degrees 52.793 minutes north and longitude 82 degrees 07.403 minutes east. This region is characterized by a tropical monsoon climate with distinct wet and dry seasons, and the native vegetation consists primarily of dry deciduous forest types adapted to seasonal water stress.

Seeds were collected from seven tropical tree species selected for their ecological and economic importance in the region. The species included Diospyros melanoxylon, commonly known as tendu or ebony, a species valued for its timber and for its leaves which are used for rolling bidis; Hardwickia binnata, a leguminous tree known for its hard wood and drought tolerance; Butea monosperma, the flame of the forest, prized for its brilliant orange-red flowers and medicinal properties; Acacia catechu, source of the valuable tannin extract known as khair; Acacia nilotica, the gum arabic tree, widely used for timber, fodder, and tannin production; Holoptelea integrifolia, the Indian elm, used in traditional medicine and for its wood; and Pithecellobium dulce, the Manila tamarind, a fast-growing tree with edible fruits and multiple uses.

After collection, seeds were carefully cleaned to remove any adhering pulp, debris, or damaged individuals. Only visually sound, well-filled seeds of uniform size were retained for the experiment. To eliminate potential surface-borne bacterial and fungal contaminants that could interfere with germination or introduce confounding variables, the seeds were subjected to surface sterilization using a 0.1 percent mercuric chloride solution. The seeds were immersed in this solution for a period of two to three minutes, with gentle agitation to ensure complete coverage of all seed surfaces. Following sterilization, the seeds were thoroughly rinsed multiple times with autoclaved distilled water to remove all traces of mercuric chloride, which could otherwise inhibit germination or cause toxicity. This rigorous sterilization protocol ensured that any observed differences in germination could be attributed to the experimental treatments rather than to pathogenic infection.

2.2 Preparation of Osmotic Solutions

Polyethylene glycol with a molecular weight of 6000 daltons, commonly referred to as polyethylene glycol 6000, was selected as the osmotic agent for simulating drought stress in this experiment. This compound is ideal for such studies because its high molecular weight prevents it from crossing plant cell membranes, ensuring that the observed effects are due to reduced external water potential rather than to direct chemical toxicity or osmotic entry into cells. The concentrations of polyethylene glycol 6000 required to achieve the desired osmotic potentials were calculated following the established and widely cited method of Michel and Kaufmann, which provides precise relationships between polyethylene glycol concentration and water potential at different temperatures.

Three levels of osmotic potential were established for this experiment. The control treatment consisted of distilled water only, corresponding to an osmotic potential of zero megapascals, representing optimal conditions for germination with no water stress. The moderate stress treatment was designed to achieve an osmotic potential of negative zero point five megapascals, representing a level of water deficit that might be encountered during mild to moderate drought conditions. The severe stress treatment was designed to achieve an osmotic potential of negative one point zero megapascals, simulating the extreme water deficit associated with severe drought. The appropriate concentrations of polyethylene glycol 6000 were dissolved in distilled water to achieve these target osmotic potentials, with careful stirring to ensure complete dissolution and uniform distribution of the polymer.

2.3 Experimental Design and Germination Setup

The experiment was established using a factorial completely randomized design, with two factors being species at seven levels and osmotic stress treatment at three levels. This design allows for the simultaneous evaluation of the main effects of species and treatment, as well as their interaction, providing insights into whether different species respond similarly or differently to the same stress levels. Each species-treatment combination was replicated four times, with each replicate consisting of twenty seeds, for a total of eighty seeds per species-treatment combination and five hundred sixty seeds per treatment level. This replication level provides adequate statistical power for detecting treatment effects and differences among species.

For each replicate, twenty seeds were placed evenly spaced on a single layer of Whatman No. 1 filter paper within a sterile glass petri dish. The filter paper was then moistened with the appropriate polyethylene glycol solution or with distilled water for the control treatment, using a sufficient volume to fully saturate the paper without causing standing water that could lead to anaerobic conditions. The petri dishes were covered with their lids to maintain high humidity and prevent evaporation, which could concentrate the solutions and alter the osmotic potential over time. The dishes were then placed in a seed germinator calibrated to maintain a constant temperature of twenty-five degrees Celsius throughout the experiment, as this temperature is optimal for germination of most tropical tree species. The germinator was also set to provide a twelve-hour photoperiod to simulate natural day-night conditions.

2.4 Germination Recording and Criteria

Germination was monitored and recorded at regular intervals following the initiation of the experiment. Observations were made at twenty-four hours, three days, and seven days after the start of the experiment, with additional observations continuing until no further germination was observed for several consecutive days. This temporal resolution allows for the assessment of both final germination percentage and germination rate, which can provide additional insights into the effects of stress on germination dynamics. At each observation time, the number of newly germinated seeds in each petri dish was counted and recorded.

The criterion for considering a seed as germinated was the visible emergence of the radicle through the seed coat, with a minimum length of two millimeters. This is a standard and widely accepted criterion in seed germination studies, as radicle emergence represents the first visible evidence that the physiological processes of germination have been successfully completed and that the seed has transitioned from a quiescent to an actively growing state. Seeds that showed signs of fungal infection or decay were noted and removed to prevent contamination of adjacent seeds, but such occurrences were minimal due to the surface sterilization protocol.

2.5 Statistical Analysis

All germination data collected during the experiment were compiled and subjected to statistical analysis to determine the significance of observed differences and to quantify the effects of the experimental factors. The primary analytical tool employed was analysis of variance, or ANOVA, which partitions the total variation in the data into components attributable to the different sources of variation, including the main effects of species and treatment, their interaction, and the random error associated with replication within treatments. The analysis was performed using the JASP statistical software package, which provides a comprehensive suite of tools for data analysis and visualization.

Before conducting the analysis of variance, the germination percentage data were checked for conformity to the assumptions of normality and homogeneity of variance, which are required for valid interpretation of the results. While germination percentages are technically binomial data, the use of analysis of variance is generally considered robust for such data when sample sizes are adequate and percentages fall within the intermediate range. The analysis of variance produced F-statistics and associated p-values for each source of variation, allowing determination of whether the observed differences were statistically significant at the conventional threshold of p less than 0.05. Where significant main effects were found, post-hoc comparisons using appropriate methods such as Tukey's honestly significant difference test were conducted to identify which specific treatment levels or species differed from each other.

3. RESULTS

3.1 Germination under Control Conditions

The results of the germination experiment revealed clear and pronounced effects of both species identity and osmotic stress level on seed germination percentage. Under the optimal conditions of the control treatment, where seeds were provided with distilled water and no osmotic stress, substantial variation was observed among the seven species in their germination success. Hardwickia binnata exhibited the highest germination percentage among all species tested, with a remarkable ninety percent of seeds successfully germinating under control conditions. This high germination rate indicates excellent seed viability and the absence of any inherent dormancy mechanisms that would prevent germination under favourable conditions. Following Hardwickia binnata, both Butea monosperma and Diospyros melanoxylon achieved germination percentages of sixty point two four percent, demonstrating good germination potential under optimal moisture availability. These values were identical for the two species, suggesting similar levels of seed viability and germination capacity.

Acacia catechu showed a germination percentage of thirty percent under control conditions, which, while lower than the previously mentioned species, still represents a reasonable level of germination success. Holoptelea integrifolia and Acacia nilotica exhibited germination percentages of fifty point eight three and fifty point eight two percent respectively, values that are nearly identical and indicate moderate germination capacity. Pithecellobium dulce achieved fifty-eight point zero one percent germination under control conditions, placing it among the moderately successful species. These control germination values provide a baseline against which the effects of osmotic stress can be assessed, and they reveal inherent differences among species in their baseline germination capacity even under optimal conditions.

3.2 Germination under Moderate Osmotic Stress

The imposition of moderate osmotic stress at the polyethylene glycol 200 level, corresponding to an osmotic potential of negative zero point five megapascals, caused a marked and significant decline in germination percentage across all seven species. However, the magnitude of this decline varied considerably among species, revealing differential sensitivity to water deficit. Hardwickia binnata maintained the highest germination under moderate stress, with sixty point eight six percent of seeds germinating. This represents a reduction from its control value of ninety percent, but the species still retained a substantial germination capacity under conditions that were inhibitory to many other species. This relatively high tolerance suggests that Hardwickia binnata possesses physiological mechanisms that allow it to maintain germination even when water availability is limited.

Butea monosperma also demonstrated relatively high tolerance to moderate stress, with thirty-six point zero four percent germination under the polyethylene glycol 200 treatment. While this represents a substantial reduction from its control value of sixty point two four percent, it still indicates that a significant proportion of seeds can successfully germinate under moderate water deficit. Acacia catechu and Diospyros melanoxylon showed germination percentages of twenty-nine point eight seven and twenty-five point two seven percent respectively under moderate stress, representing reductions of approximately fifty to sixty percent from their control values. These species appear to have intermediate sensitivity to water stress, with some capacity for germination under moderate deficit but with substantial inhibition compared to optimal conditions.

Holoptelea integrifolia and Acacia nilotica exhibited much more dramatic reductions in germination under moderate stress, falling to only five point three five and seven point four eight percent respectively. These extremely low values represent near-complete inhibition of germination for these species, indicating high sensitivity to even moderate levels of water deficit. The most extreme response was observed in Pithecellobium dulce, which showed complete inhibition of germination under the polyethylene glycol 200 treatment, with no seeds germinating at this stress level. This complete failure to germinate under moderate stress reveals extreme sensitivity and suggests that this species requires near-optimal moisture conditions for successful germination.

3.3 Germination under Severe Osmotic Stress

The imposition of severe osmotic stress at the polyethylene glycol 300 level, corresponding to an osmotic potential of negative one point zero megapascals, resulted in complete or near-complete inhibition of germination in all species tested. Hardwickia binnata and Butea monosperma were the only species that retained any measurable germination under this extreme stress level, with minimal germination percentages of five point three five and six point six four percent respectively. These low values, while representing successful germination of a small proportion of seeds, still indicate that the vast majority of seeds were unable to germinate under severe water deficit. For all other species, including Acacia catechu, Acacia nilotica, Holoptelea integrifolia, Diospyros melanoxylon, and Pithecellobium dulce, germination was completely inhibited under the severe stress treatment, with zero percent germination recorded.

The complete inhibition of germination in most species under severe stress demonstrates that an osmotic potential of negative one point zero megapascals represents a threshold beyond which the physiological processes necessary for germination cannot be initiated or completed in these tropical tree species. Even for the relatively tolerant Hardwickia binnata and Butea monosperma, germination at this stress level was so low as to be essentially negligible from a practical standpoint for population establishment. These results highlight the extreme vulnerability of the germination phase to severe drought stress and underscore the importance of adequate soil moisture for successful seedling recruitment.

3.4 Statistical Analysis Results

The statistical analysis using analysis of variance confirmed the significance of the observed patterns and provided quantitative measures of the relative importance of different sources of variation. The treatment effect, representing the influence of osmotic stress level on germination, was highly significant with a p-value less than 0.001, confirming that water availability has a profound and statistically robust effect on germination success across all species. The magnitude of this effect, as measured by the partial eta squared statistic, was 0.888, indicating that treatment alone accounted for nearly eighty-nine percent of the total variance in the data. This remarkably high value underscores the dominant role of water availability as the primary environmental factor governing seed germination in these tropical tree species.

The species effect was also highly significant with a p-value less than 0.001, confirming that there are inherent genetic differences among the seven species in their germination capacity and response to water stress. This significant species effect validates the observation that some species, such as Hardwickia binnata and Butea monosperma, are inherently more tolerant of water deficit than others, such as Pithecellobium dulce and Holoptelea integrifolia. The species by treatment interaction effect was likewise highly significant with a p-value less than 0.001, indicating that different species respond differently to the same levels of osmotic stress. This significant interaction confirms that the ranking of species in terms of germination success changes depending on the stress level, with some species showing proportionally greater declines under stress than others.

Post-hoc comparisons among the three treatment levels revealed that all pairwise differences were statistically significant at the p less than 0.001 level. Germination under the control treatment was significantly higher than under moderate stress, which was in turn significantly higher than under severe stress. This confirms that even the moderate stress level imposes a significant challenge to germination, and that the severe stress level represents an even greater impediment. The consistent ordering of treatment effects across species, despite the significant interaction, indicates that while the magnitude of response varies among species, the direction of response is uniform, with increasing stress always leading to reduced germination.

4. DISCUSSION

4.1 The Sensitivity of Seed Germination to Water Stress

The results of this study provide compelling evidence that seed germination represents one of the most sensitive phases of the plant life cycle, highly vulnerable to even moderate reductions in water availability. The dramatic decline in germination observed under both moderate and severe osmotic stress across all seven species confirms that water deficit during this critical stage can severely compromise recruitment potential, with cascading effects on population dynamics and community composition. This finding is consistent with a large body of literature documenting the sensitivity of germination to water stress across diverse plant groups and ecological contexts. As Ashraf and colleagues have noted, seed germination represents the most sensitive phase of the plant life cycle, during which environmental stresses can have disproportionately large impacts on subsequent survival and reproduction.

The physiological basis for this sensitivity lies in the fundamental requirements of the germination process. The earliest event in germination is water imbibition, the physical uptake of water by dry seeds, which is driven by the water potential gradient between the seed and its surrounding environment. When external water potential is reduced by osmotic stress, this gradient is diminished, slowing or preventing the imbibition necessary to rehydrate seed tissues and activate metabolism. This initial impairment then cascades through subsequent physiological processes, disrupting enzyme activation, reducing the hydrolysis and mobilization of stored seed reserves, and ultimately preventing radicle emergence. As Ahmad and Bano observed, the earliest physiological impairment under water stress is a reduction in water imbibition, which then disrupts the entire suite of metabolic processes required for successful germination.

Beyond the initial inhibition of imbibition, osmotic stress can also directly interfere with the mobilization of stored reserves that provide the energy and building blocks for seedling growth. Lin and Kao demonstrated that in many species, osmotic stress constrains the hydrolysis of starches and proteins in the endosperm or cotyledons, limiting the availability of sugars and amino acids for the growing embryo. This constraint on reserve mobilization can delay or prevent radicle emergence even if initial imbibition has occurred, contributing to the reduced germination percentages observed under stress conditions. The combined effects of reduced imbibition and impaired reserve mobilization create a physiological bottleneck that many seeds cannot overcome when water availability is limited.

Among environmental factors affecting plant establishment, water availability plays a particularly critical role, influencing both the probability of germination and the subsequent success of seedling growth. Luo and colleagues have emphasized that water availability is perhaps the single most important environmental factor governing germination timing and success in most terrestrial ecosystems. The present results strongly support this view, with treatment alone accounting for nearly ninety percent of the variance in germination data. This overwhelming influence of water availability highlights the vulnerability of forest regeneration to changes in precipitation patterns and the importance of maintaining adequate soil moisture during germination windows.

4.2 Species-Specific Drought Tolerance Strategies

The highly significant species effect and species by treatment interaction observed in this study reveal that drought tolerance is not a uniform trait but rather varies considerably among species, reflecting diverse evolutionary strategies for coping with water deficit. This inter- and intra-specific genetic variability in drought tolerance has been documented across numerous plant groups and represents a valuable resource for identifying genotypes with enhanced resilience to water stress. As Lin and colleagues and Gaspar and colleagues have noted, understanding and harnessing this genetic variability is essential for developing climate-resilient plant materials for forestry, agriculture, and ecological restoration.

The relatively higher tolerance exhibited by Hardwickia binnata and Butea monosperma under both moderate and severe stress suggests that these species possess physiological mechanisms that allow them to maintain metabolic activity and radicle emergence even when water availability is limited. These mechanisms may include more efficient water uptake systems, the ability to maintain enzyme activity at reduced tissue water potentials, or more robust systems for protecting cellular structures from dehydration damage. The identification of these relatively tolerant species is particularly valuable for restoration practitioners working in drought-prone areas, as they represent candidate species that may be more likely to establish successfully under marginal moisture conditions.

In contrast, the extreme sensitivity of Pithecellobium dulce, which failed to germinate even under moderate stress, indicates that this species requires near-optimal moisture conditions for successful germination. Such species may be more vulnerable to climate change impacts and may require targeted conservation interventions, such as planting in particularly moist microsites or supplementing with irrigation during establishment. The intermediate responses of species like Acacia catechu and Diospyros melanoxylon suggest moderate tolerance that may be adequate under some conditions but insufficient under severe or prolonged drought.

The diversity of responses observed among these seven co-occurring tropical tree species highlights the importance of species-specific knowledge for effective forest management and restoration. A one-size-fits-all approach to species selection, assuming that all native species are equally suitable for planting in drought-prone areas, would be misguided and could lead to restoration failures. Instead, restoration planners should consider the specific tolerance characteristics of different species and match them appropriately to site conditions, selecting more tolerant species for the most water-limited locations and reserving more sensitive species for areas with more reliable moisture.

4.3 Utility of Polyethylene Glycol for Drought Tolerance Screening

The successful use of polyethylene glycol 6000 to create precise and reproducible osmotic stress gradients in this study confirms the value of this technique for early screening of drought tolerance in tree species. As numerous researchers have demonstrated, polyethylene glycol-induced osmotic stress provides a controlled and standardized method for evaluating germination responses to water deficit, enabling the identification of tolerant genotypes without the confounding effects of soil variability or other environmental factors present in field trials. This laboratory-based screening approach is particularly valuable for its cost-effectiveness and time efficiency, allowing large numbers of species, provenances, or individual genotypes to be evaluated relatively quickly.

Singh and Ansari have emphasized that germination tests conducted under polyethylene glycol-induced stress remain valuable for identifying provenances and genotypes capable of rapid establishment under limited soil moisture, provided that careful attention is paid to standardizing seed quality and accounting for potential confounding variables. The present study, by using seeds collected from a single location and processed uniformly, minimized variation in seed quality that could otherwise obscure true genetic differences in drought tolerance. This careful standardization strengthens confidence that the observed differences among species reflect inherent physiological differences rather than artifacts of seed handling or storage.

The dose-response relationship observed in this study, with increasingly severe stress causing progressively greater inhibition of germination, validates the use of multiple polyethylene glycol concentrations to characterize the full range of species responses. By testing at both moderate and severe stress levels, we were able to distinguish species that show gradual decline in germination with increasing stress from those that exhibit threshold responses, where germination collapses once a critical stress level is exceeded. This more nuanced characterization of drought tolerance is essential for predicting species performance under different drought scenarios and for selecting appropriate species for sites with different levels of water limitation.

The complete inhibition of germination observed in most species at the severe stress level of negative one point zero megapascals is consistent with findings from other woody species. Rahemi and colleagues studying four wild almond species observed progressive decreases in germination capability and rate with increasing polyethylene glycol concentrations, and noted that high stress levels could extend the critical early stage of seedling establishment, impacting survival and growth in natural settings. Similarly, Rodrigues and colleagues found that germination of Caesalpinia echinata decreased notably with more negative water potentials, with complete cessation at higher stress levels. These convergent findings across diverse woody species underscore the universal vulnerability of seed germination to severe water deficit and highlight the importance of maintaining adequate soil moisture during this critical phase.

4.4 Implications for Forest Restoration and Management

The findings of this study have direct and practical implications for forest restoration and management in drought-prone regions, particularly in the tropical dry forests of India and similar ecosystems elsewhere. The identification of Hardwickia binnata and Butea monosperma as relatively drought-tolerant during germination suggests that these species should be prioritized for planting programs in areas where water availability during the establishment phase is likely to be limiting. Both species are native to the region and well-adapted to local conditions, making them ecologically appropriate choices for restoration efforts. Their relatively high germination under moderate stress indicates that they are more likely to establish successfully under marginal moisture conditions, potentially reducing the need for costly irrigation or repeat planting.

Conversely, the extreme sensitivity of Pithecellobium dulce to even moderate stress suggests that this species may be a poor choice for planting in water-limited environments unless supplemental irrigation can be provided. In restoration contexts where resources for post-planting care are limited, selecting species with greater inherent drought tolerance may be essential for achieving acceptable establishment rates. The intermediate responses of species like Acacia catechu and Diospyros melanoxylon indicate that they may be suitable for sites with moderate moisture availability but may struggle under more severe drought conditions. This graded understanding of species tolerances allows for more nuanced and targeted species selection, matching each species to the sites where it is most likely to succeed.

Beyond species selection, the results also highlight the importance of considering germination requirements in the timing of restoration activities. The dramatic inhibition of germination under severe stress suggests that planting should be timed to coincide with periods of reliable moisture, typically early in the rainy season when soil moisture is high and the probability of subsequent drought is low. In years with below-average rainfall or delayed onset of rains, it may be advisable to postpone planting rather than risk high mortality due to germination failure. This flexible, adaptive approach to restoration timing can help maximize the success of planting efforts despite interannual climate variability.

4.5 Limitations and Future Research Directions

While this study provides valuable insights into species-specific drought tolerance during germination, several limitations should be acknowledged and addressed in future research. First, the study examined only the germination phase, and does not provide information on how the different species respond to drought during subsequent seedling growth and establishment. It is possible that species showing relatively high germination tolerance may be more sensitive to drought during later stages, or vice versa. Future studies should extend the observation period to include early seedling growth, measuring parameters such as radicle and plumule length, biomass accumulation, and physiological indicators of stress.

Second, the study was conducted under controlled laboratory conditions using polyethylene glycol to simulate drought stress. While this approach offers excellent control and reproducibility, it may not fully capture the complexity of drought stress in natural soils, where factors such as soil texture, hydraulic conductivity, and microbial interactions can modulate the actual water availability experienced by seeds. Future research should include field validation studies, comparing germination and establishment of the different species under natural drought conditions to confirm the laboratory findings.

Third, the study examined only seven species, representing a small fraction of the diverse tree flora of tropical dry forests. Expanding the screening to include additional species, particularly those that are rare, threatened, or of special conservation concern, would provide a more comprehensive understanding of drought tolerance patterns and help identify additional species suitable for restoration. Fourth, the study did not investigate the physiological and biochemical mechanisms underlying the observed differences in drought tolerance. Future research could explore parameters such as osmotic adjustment capacity, antioxidant enzyme activity, and accumulation of stress-related proteins to understand why some species are more tolerant than others.

Fifth, the study used seeds collected from a single population of each species, and does not address the possibility of within-species genetic variation in drought tolerance. Provenance trials, testing seeds from multiple populations across the species' geographic range, could identify particularly drought-tolerant genotypes within species and provide guidance for selecting seed sources for restoration in different environments. Such studies would also help predict how species might respond to climate change through local adaptation and genetic differentiation.

5. CONCLUSION

This comprehensive study of seed germination under polyethylene glycol-induced osmotic stress has provided valuable insights into the drought tolerance characteristics of seven important tropical tree species native to the dry deciduous forests of India. The results clearly demonstrate that water availability is the dominant environmental factor governing germination success, accounting for nearly ninety percent of the observed variation in the data. This overwhelming influence underscores the vulnerability of the germination phase to drought stress and highlights the importance of maintaining adequate soil moisture for successful seedling recruitment in forest ecosystems.

The study revealed pronounced species-specific differences in drought tolerance during germination, ranging from the relatively high tolerance of Hardwickia binnata and Butea monosperma to the extreme sensitivity of Pithecellobium dulce, which failed to germinate even under moderate stress. These differences reflect diverse evolutionary strategies for coping with water deficit and have direct implications for species selection in afforestation and restoration programs. The relatively higher tolerance of Hardwickia binnata and Butea monosperma under moderate stress suggests their potential suitability for planting in drought-prone regions where water availability during establishment is often limited. In contrast, the extreme sensitivity of Pithecellobium dulce indicates that this species may require more favourable moisture conditions and may be less suitable for planting in water-limited environments without supplemental irrigation.

The significant species by treatment interaction confirms that different species respond differently to the same levels of stress, emphasizing the need for species-specific knowledge in restoration planning. A nuanced understanding of these differential responses allows for more targeted species selection, matching each species to the sites where it is most likely to succeed. The dose-response relationship observed, with increasingly severe stress causing progressively greater inhibition, provides a basis for predicting species performance under different drought scenarios and for setting thresholds for restoration timing.

The successful application of polyethylene glycol-induced osmotic stress as a screening tool in this study confirms its utility for early identification of drought-tolerant genotypes. This cost-effective and time-efficient approach can be readily applied to additional species and populations, building a comprehensive database of drought tolerance characteristics to support climate-resilient forest management. As climate change increases the frequency and intensity of drought events in many tropical regions, such information will become increasingly valuable for guiding species selection, designing restoration projects, and targeting conservation efforts to the most vulnerable species.

In conclusion, this study contributes fundamental knowledge about the germination ecology of tropical tree species under water stress, providing a scientific basis for selecting climate-resilient species for sustainable forest management, afforestation, and restoration programs. The relatively higher tolerance of Hardwickia binnata and Butea monosperma makes them promising candidates for planting in drought-prone areas, while the sensitivity of other species highlights the need for careful site-species matching and, where necessary, supplemental interventions to ensure successful establishment. By integrating this knowledge into restoration planning, practitioners can enhance the resilience of restored forests to climate change and contribute to the long-term conservation of tropical forest ecosystems.

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