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IMPACT OF MANNITOL ON GERMINATION BEHAVIOR AND SEEDLING DEVELOPMENT OF MOTH BEAN

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Abstract

Moth bean (*Vigna aconitifolia*) is an important drought-resilient legume cultivated in arid and semi-arid regions, where water scarcity frequently affects seed germination and early seedling establishment. The present study evaluates the effect of mannitol-induced osmotic stress on germination behavior and early seedling development of moth bean under controlled laboratory conditions. Mannitol was used as a non-ionic osmoticum to simulate drought stress, and its impact was assessed through germination percentage, seedling length, and seedling biomass at 96 and 144 hours after sowing.

Seeds of two moth bean varieties (RMO-257 and RMO-435) were subjected to increasing concentrations of mannitol (0.05–0.5 M), along with a control. Germination percentage was recorded, and seedling growth was evaluated by measuring radicle length, hypocotyl length, total seedling length, and final seedling weight. All observations were recorded in three replications and expressed as mean \pm standard error (SE). The results showed a concentration-dependent decline in germination percentage and seedling growth parameters. Higher mannitol concentrations caused substantial inhibition of both seedling elongation and biomass accumulation, with more pronounced effects at 144 hours compared to 96 hours.

The findings demonstrate that mannitol-induced osmotic stress significantly impairs germination and early seedling development in moth bean. Although moth bean is generally regarded as drought tolerant, the early developmental stages remain highly sensitive to osmotic stress. This study provides valuable physiological insights into drought stress responses in moth bean and supports early-stage screening for stress tolerance.

Keywords: Moth Bean; Mannitol; Osmotic Stress; Seed Germination; Seedling Growth

1. Introduction

Moth bean (*Vigna aconitifolia* (Jacq.) Marechal) is a highly valued leguminous crop extensively cultivated in arid and semi-arid regions of South Asia, particularly in areas characterized by erratic rainfall, high temperature, and poor soil fertility. Owing to its inherent adaptability to harsh agro-climatic conditions, moth bean serves as an important component of subsistence farming systems and contributes significantly to regional food security. In addition to its nutritional value as a protein-rich pulse, moth bean also plays a crucial role in fodder supply and soil fertility enhancement through biological nitrogen fixation, thereby supporting sustainable agricultural practices in marginal environments.

Despite its reputation as a drought-resilient crop, the success of moth bean cultivation is strongly influenced by effective seed germination and early seedling establishment. These early developmental stages represent critical bottlenecks in the crop life cycle, as they are highly sensitive to fluctuations in soil moisture availability. In rainfed ecosystems, inadequate or irregular rainfall during sowing often results in delayed germination, poor seedling vigor, and uneven crop stands, ultimately leading to reduced productivity. Therefore, understanding the response of moth bean to water stress during early growth stages is essential for improving crop establishment under water-limited conditions.

Seed germination is a complex physiological process that begins with water uptake (imbibition) and is followed by the activation of metabolic pathways necessary for reserve mobilization, enzyme synthesis, and cell division. Adequate moisture availability is a prerequisite for these processes. Under drought conditions, reduced water potential restricts seed imbibition, delays enzymatic activation, and suppresses radicle protrusion, resulting in decreased germination percentage and delayed emergence. Such stress-induced inhibition of germination has long-term consequences for plant growth and yield potential.



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Early seedling growth is equally vulnerable to water deficit stress. Osmotic stress limits cell elongation by reducing turgor pressure, interferes with hormonal regulation of growth, and disrupts nutrient uptake and translocation. These physiological constraints lead to reduced root and shoot development, diminished biomass accumulation, and compromised seedling vigor. Since early root establishment is crucial for subsequent water and nutrient acquisition, impairment at this stage can severely affect plant survival and performance under prolonged drought conditions.

In experimental studies, mannitol is widely used as a non-ionic osmoticum to simulate drought stress under controlled laboratory conditions. Mannitol lowers the water potential of the growth medium without introducing ionic toxicity, allowing researchers to isolate the effects of osmotic stress from those of salinity. This approach provides a reliable and reproducible method for evaluating plant responses to water deficit during germination and early growth stages. Mannitol-induced osmotic stress has been successfully employed to assess drought tolerance in several crop species, particularly legumes, by examining changes in germination behavior and seedling growth parameters.

Although previous studies have documented the effects of osmotic stress on seed germination and early growth in various legumes, comprehensive and stage-specific information on moth bean remains limited. Moreover, comparative assessments of early seedling responses at different developmental stages are scarce, despite their importance in understanding stress progression and cumulative effects. Addressing this knowledge gap is critical for identifying sensitive growth phases and developing strategies for improving drought tolerance during crop establishment.

In this context, the present study aims to evaluate the impact of mannitol-induced osmotic stress on germination percentage, seedling length, and seedling biomass of moth bean at two early developmental stages, namely 96 and 144 hours after sowing. By examining both germination behavior and post-germinative growth responses, the study seeks to provide a comprehensive understanding of early-stage osmotic stress effects. The findings are expected to contribute valuable insights into drought stress physiology of moth bean and support future efforts in genotype screening and management strategies for cultivation under water-limited environments.

2. Materials and Methods

2.1 Experimental Material

The experiment was conducted using healthy, uniform, and viable seeds of two moth bean (*Vigna aconitifolia* (Jacq.) Marechal) varieties, namely **RMO-257** and **RMO-435**. These varieties were selected due to their agronomic relevance and contrasting growth performance under stress conditions. Prior to experimentation, seeds were carefully examined and sorted to ensure uniformity in size, color, and shape. Seeds showing visible signs of damage, shrivelling, discoloration, or fungal infection were discarded to avoid experimental bias and ensure consistency across treatments.

Before imposing osmotic stress treatments, the selected seeds were surface-cleaned to remove adhering dust and contaminants. All experimental procedures were conducted under laboratory conditions to minimize environmental variability and ensure controlled exposure to stress treatments.

2.2 Osmotic Stress Treatments

Osmotic stress was simulated using aqueous solutions of **mannitol**, a non-ionic and non-toxic osmoticum commonly employed to mimic drought conditions in plant physiological studies. Mannitol was chosen because it reduces the water potential of the germination medium without causing ionic imbalance, thereby allowing accurate assessment of osmotic stress effects.

A range of mannitol concentrations was prepared to impose graded levels of osmotic stress. The treatments included **0.05 M, 0.10 M, 0.15 M, 0.20 M, 0.25 M, 0.30 M, 0.40 M, and 0.50 M mannitol**, along with a **control treatment** consisting



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of distilled water. These concentrations were selected to represent mild to severe osmotic stress levels and to evaluate dose-dependent responses of germination and early seedling growth.

All solutions were freshly prepared using analytical-grade mannitol and distilled water to ensure accuracy and consistency. Seeds of both varieties were exposed to each treatment under identical experimental conditions.

2.3 Germination Test

The germination test was conducted under controlled laboratory conditions using a standard germination protocol. Seeds from each treatment were placed in suitable germination containers lined with moist germination paper or filter paper, which was uniformly wetted with the respective mannitol solution or distilled water for the control.

The seeds were incubated at room temperature under natural photoperiod conditions. Adequate care was taken to maintain consistent moisture levels by periodically adding the respective solutions as required. Germination was monitored daily, and a seed was considered germinated when visible radicle protrusion was observed.

Germination percentage was calculated after germination had stabilized across all treatments using the following formula:

$$\text{Germination Percentage (\%)} = \frac{\text{Number of seeds germinated}}{\text{Total number of seeds sown}} \times 100$$

This parameter was used as an indicator of the seed's ability to initiate growth under osmotic stress conditions.

2.4 Seedling Growth Measurements

Seedling growth parameters were recorded at two early developmental stages, **96 hours** and **144 hours** after sowing, to assess both early and slightly advanced growth responses to osmotic stress.

At each observation interval, randomly selected seedlings from each treatment and variety were carefully removed and washed to remove adhering solution without damaging the tissues. The following growth parameters were measured:

- **Radicle length (cm):** Measured from the point of radicle emergence to the tip of the primary root using a graduated scale.
- **Hypocotyl length (cm):** Measured from the cotyledonary node to the base of the radicle.
- **Total seedling length (cm):** Calculated as the sum of radicle and hypocotyl lengths.
- **Final seedling weight (mg):** Seedlings were gently blotted to remove surface moisture and weighed using an electronic balance to determine fresh biomass.

These parameters were selected because they provide reliable indicators of seedling vigor, growth potential, and physiological performance under stress conditions.

2.5 Statistical Analysis

All experimental treatments were arranged with **three replications**, and data were recorded separately for each moth bean variety. The observed values for germination percentage, seedling length, and seedling weight were expressed as **mean ± standard error (SE)**.

The use of mean ± SE provides an estimate of central tendency and variability, enabling comparison of treatment effects across stress levels and developmental stages. This statistical representation is commonly employed in plant stress



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physiology studies and is appropriate for evaluating trends and treatment responses under controlled experimental conditions.

Methodological Rigor Statement

The experimental design, stress simulation approach, and measurement protocols employed in this study were selected to ensure reproducibility, accuracy, and reliability of results. All procedures were performed uniformly across treatments to minimize experimental error and allow meaningful interpretation of osmotic stress effects on moth bean germination and early seedling development.

3. Results

3.1 Effect of Mannitol on Germination Percentage

Increasing concentrations of mannitol induced a clear and progressive decline in germination percentage in both moth bean varieties, indicating a concentration-dependent inhibitory effect of osmotic stress on seed germination (Table 1). Under control conditions, seeds of both RMO-257 and RMO-435 exhibited 100% germination, reflecting optimal hydration and favorable conditions for metabolic activation and radicle emergence.

At lower mannitol concentrations, germination remained largely unaffected, suggesting that both varieties possess a certain degree of tolerance to mild osmotic stress. However, with further increases in mannitol concentration, a gradual reduction in germination percentage was observed, indicating increasing sensitivity of the germination process to reduced water potential. This decline became more pronounced at higher stress levels, where limited water availability likely restricted seed imbibition and delayed the initiation of enzymatic and metabolic processes essential for germination.

At the highest mannitol concentration (0.5 M), germination percentage decreased substantially, reaching 70% in variety RMO-257 and 60% in variety RMO-435. The greater reduction observed in RMO-435 suggests a comparatively higher sensitivity to severe osmotic stress during germination. This varietal difference highlights the presence of genotypic variability in stress response, which may be associated with differences in seed coat permeability, reserve mobilization efficiency, or stress tolerance mechanisms.

Overall, the results demonstrate that while moth bean seeds are capable of germinating under mild osmotic stress, severe mannitol-induced stress significantly suppresses germination. The concentration-dependent decline in germination percentage underscores the vulnerability of the germination phase to water deficit conditions and emphasizes the importance of adequate moisture availability during sowing for successful crop establishment.

Table 1. Effect of mannitol on germination percentage of moth bean seeds

Values are mean percentages.

Treatment	RMO-257	RMO-435
Control	100%	100%
0.05 M	100%	100%
0.1 M	100%	90%
0.15 M	90%	95%
0.2 M	90%	90%
0.25 M	90%	90%
0.3 M	90%	90%



Treatment	RMO-257	RMO-435
0.4 M	80%	90%
0.5 M	70%	60%

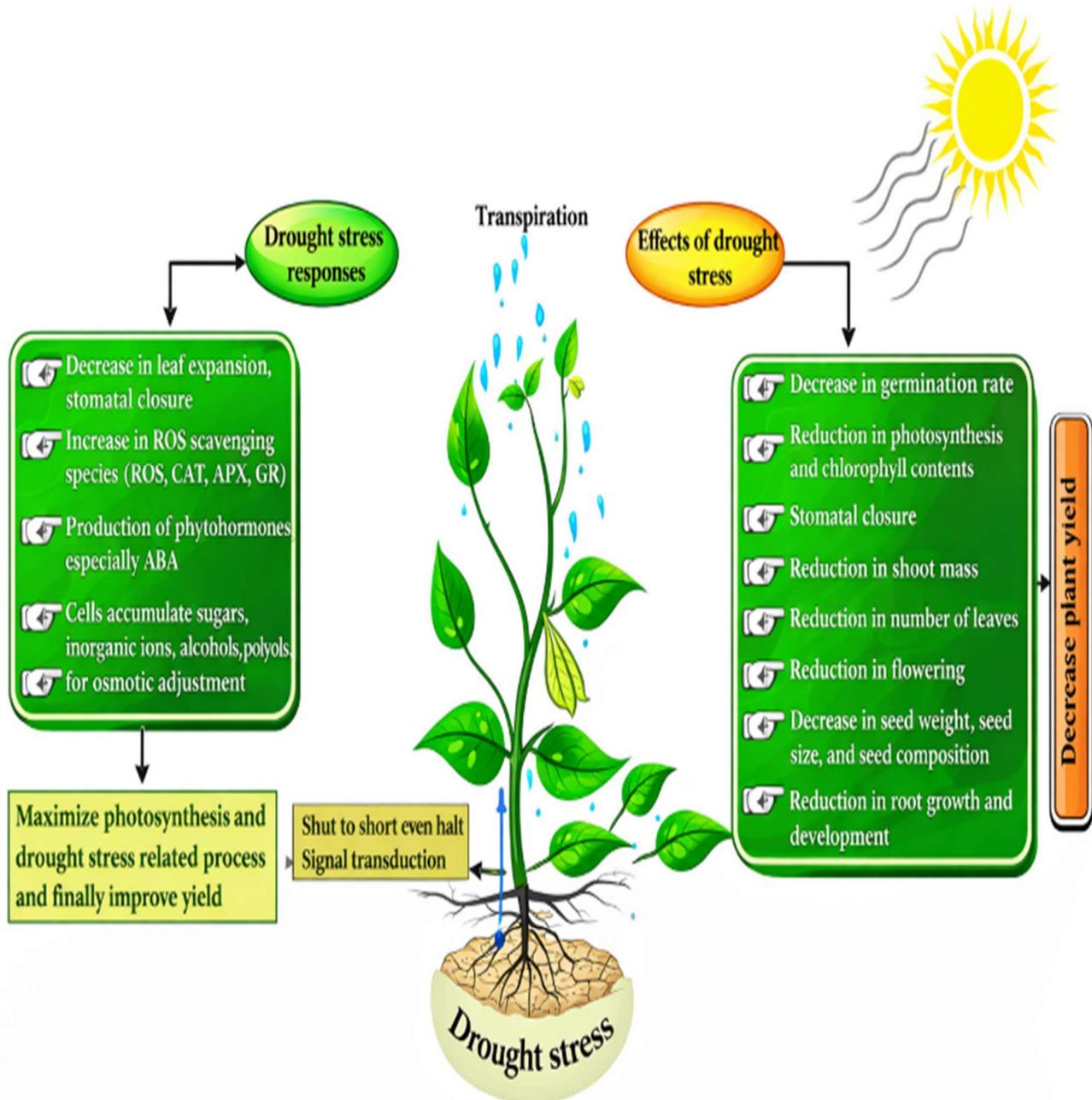


Figure 1. Effect of increasing mannitol concentration on germination percentage of moth bean.



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3.2 Seedling Length after 96 Hours

Seedling length showed a marked and progressive decline with increasing levels of mannitol-induced osmotic stress in both moth bean varieties (Table 2). Under control conditions, seedlings exhibited maximum growth, characterized by well-developed radicle and hypocotyl elongation, indicating optimal water availability and unhindered cellular expansion during early growth.

At lower concentrations of mannitol, a moderate reduction in seedling length was observed, suggesting that mild osmotic stress partially restricted growth but did not completely inhibit elongation processes. However, as mannitol concentration increased, a substantial reduction in total seedling length became evident. This decline was primarily attributed to severe suppression of both radicle and hypocotyl elongation under higher stress levels.

The pronounced reduction in radicle length under osmotic stress indicates impaired root growth, which may result from reduced cell division and elongation due to limited turgor pressure. Since the radicle is the primary organ responsible for water and nutrient uptake during early development, its inhibition reflects a critical stress-induced limitation. Similarly, reduced hypocotyl elongation under higher mannitol concentrations suggests disrupted shoot growth, likely caused by restricted cell expansion and altered hormonal regulation under low water potential conditions.

Overall, the results demonstrate that increasing osmotic stress severely constrains early seedling growth by limiting elongation of both root and shoot components. The sensitivity of seedling length to mannitol stress highlights the vulnerability of early growth stages in moth bean and underscores the importance of adequate moisture availability for successful seedling establishment.

Table 2. Seedling length (cm) after 96 hours under mannitol stress

Treatment	RMO-257 Total	RMO-435 Total
Control	4.81	2.95
0.05 M	4.58	2.81
0.1 M	4.21	2.63
0.15 M	3.94	2.41
0.2 M	3.62	2.26
0.25 M	3.28	2.04
0.3 M	3.01	1.92
0.4 M	2.64	1.75
0.5 M	1.98	1.54

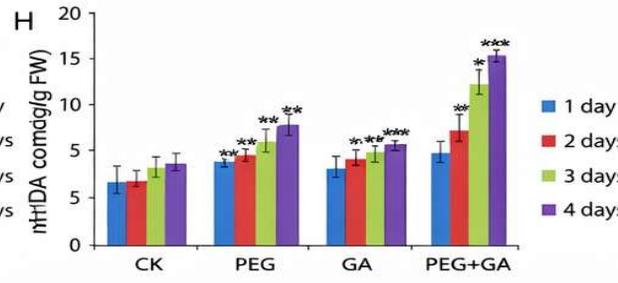
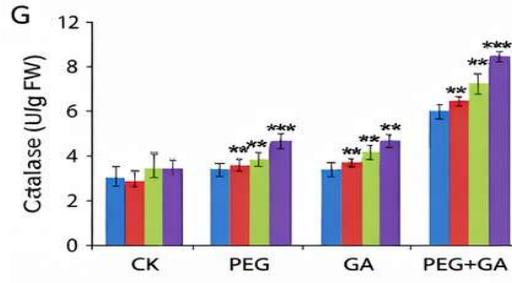
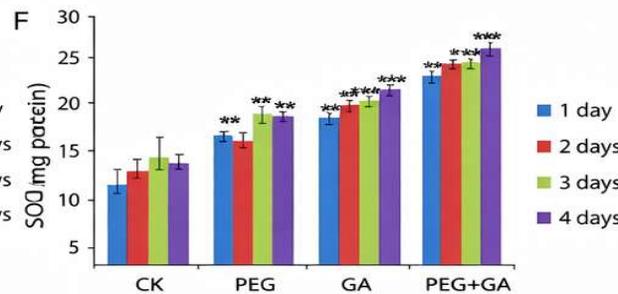
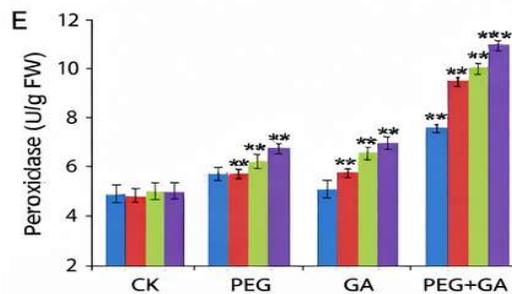
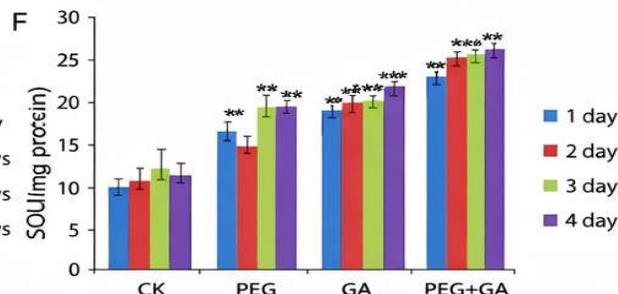
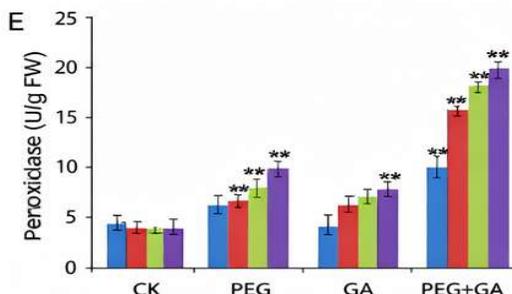
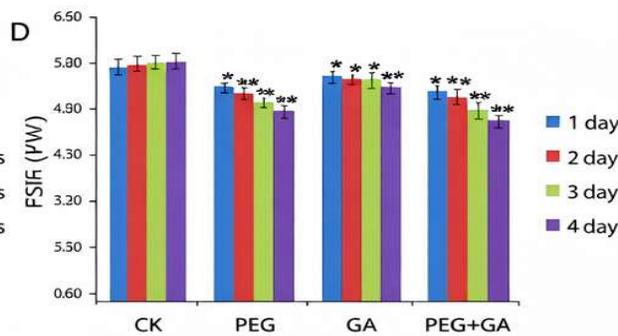
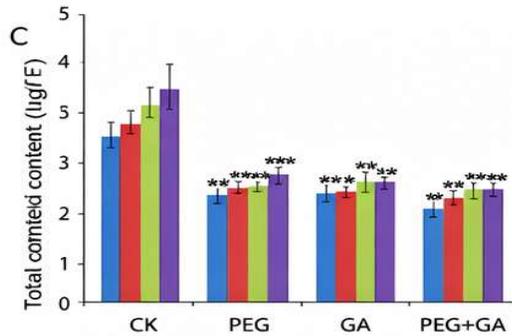
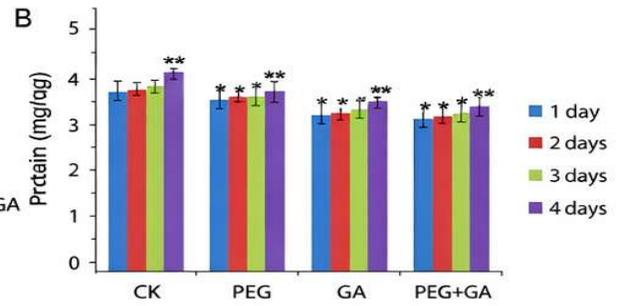
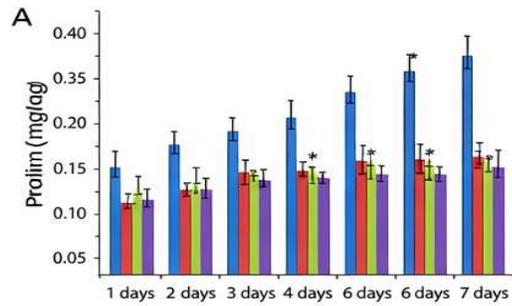




Figure 2. Effect of mannitol-induced osmotic stress on seedling length of moth bean after 96 hours.

3.3 Seedling Growth and Biomass after 144 Hours

At 144 hours after sowing, the inhibitory effects of mannitol-induced osmotic stress on seedling growth became more pronounced in both moth bean varieties, indicating a cumulative impact of prolonged stress exposure (Tables 3 and 4). Compared to the observations recorded at 96 hours, seedlings subjected to extended osmotic stress exhibited markedly reduced growth performance, reflecting sustained physiological constraints under low water potential conditions.

Seedling length declined progressively with increasing mannitol concentration, with control seedlings attaining the maximum elongation. In contrast, seedlings exposed to higher concentrations of mannitol showed substantial suppression of both radicle and hypocotyl growth, resulting in significantly reduced total seedling length. This reduction suggests that prolonged osmotic stress restricts cell elongation by maintaining low turgor pressure and limiting the expansion of growing tissues over time.

Similarly, final seedling weight measured at 144 hours showed a significant decline under osmotic stress. Control seedlings accumulated greater biomass, whereas stressed seedlings exhibited reduced weight, particularly at higher mannitol concentrations. The reduction in biomass accumulation indicates impaired dry matter synthesis and limited mobilization of seed reserves under prolonged water-deficit conditions. Continuous stress exposure likely disrupts metabolic processes, reduces cellular growth efficiency, and suppresses tissue development.

The greater severity of growth inhibition observed at 144 hours compared to earlier stages highlights the time-dependent nature of osmotic stress effects. While seedlings may initially tolerate mild stress, sustained exposure leads to cumulative physiological damage that further compromises growth and biomass production. These findings emphasize that early seedling stages of moth bean are highly sensitive to prolonged osmotic stress, which may adversely affect subsequent growth and establishment under drought-prone conditions.

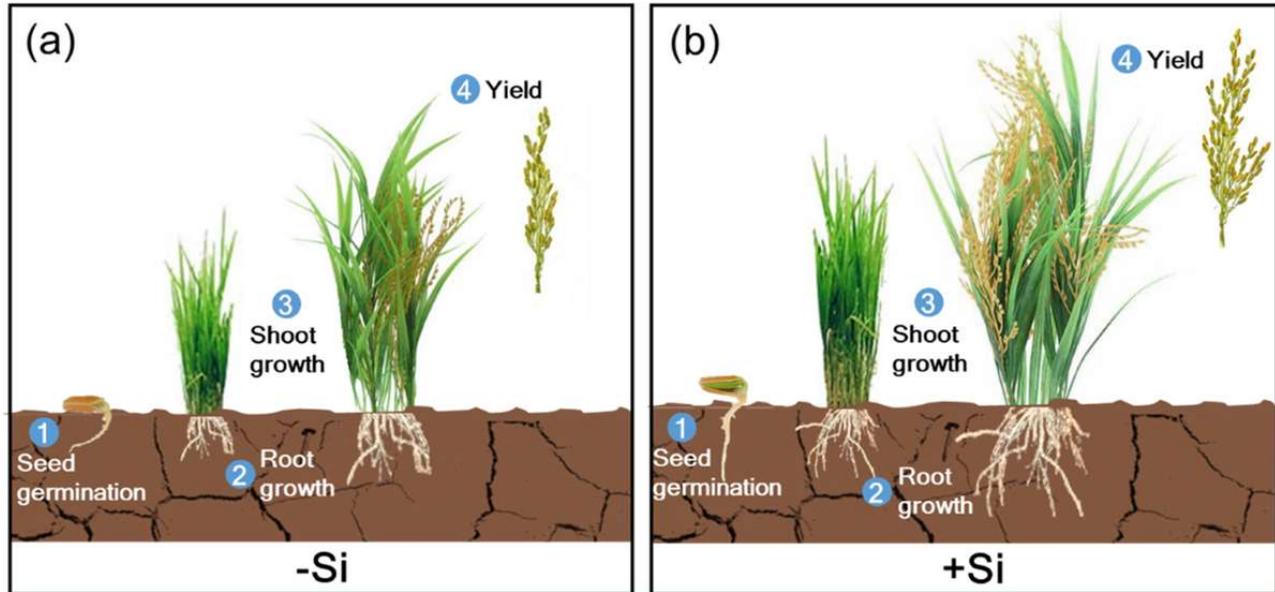


Table 3. Seedling length (cm) after 144 hours

Treatment	RMO-257 Total	RMO-435 Total
Control	7.24	6.81
0.05 M	6.62	6.14
0.1 M	6.03	5.72
0.15 M	5.48	5.01
0.2 M	4.92	4.46
0.25 M	4.31	3.92
0.3 M	3.87	3.48
0.4 M	3.21	2.94
0.5 M	2.69	2.43

Table 4. Final seedling weight (mg) after 144 hours

Treatment	RMO-257	RMO-435
Control	125.8	124.7
0.05 M	104.4	111.0
0.1 M	94.6	105.9
0.15 M	104.2	78.5
0.2 M	98.5	76.9
0.25 M	76.0	84.7
0.3 M	82.1	83.6
0.4 M	82.7	76.5
0.5 M	69.6	65.9



- (c) Si application under drought stress
- ① Stimulating seed germination
 - ② Increasing root length, root surface area and volume, root biomass
 - ③ Improving plant height, leaf area index, shoot biomass
 - ④ Promoting plant biomass, panicle/spike length and weight, number of tillers, yield

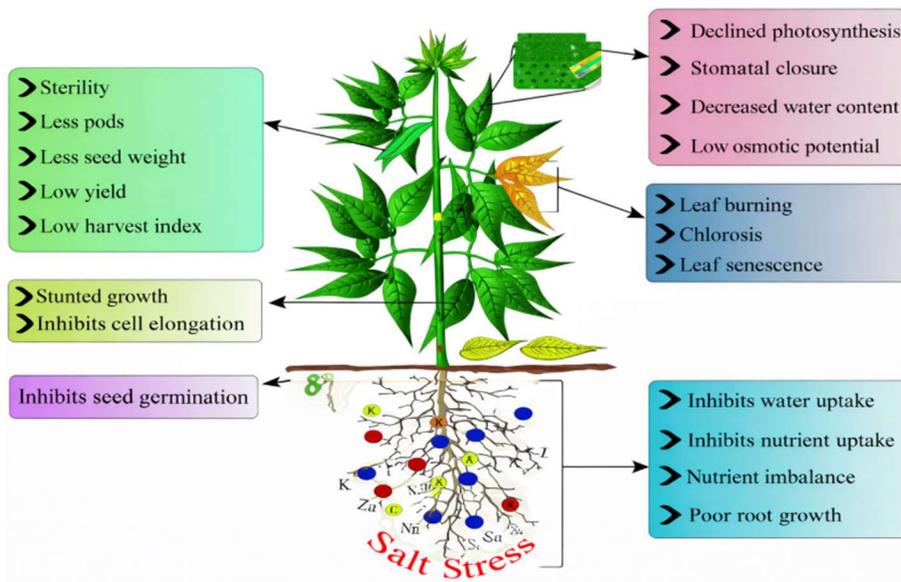


Figure 3. Effect of mannitol-induced osmotic stress on seedling biomass of moth bean after 144 hours.



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4. Discussion

The observed reduction in germination percentage and seedling growth parameters under mannitol-induced osmotic stress can be primarily attributed to restricted water uptake and subsequent impairment of key metabolic activities. Mannitol lowers the external water potential of the germination medium, thereby limiting seed imbibition, which is the initial and essential step for triggering germination. Insufficient water availability delays the activation of hydrolytic enzymes responsible for mobilizing stored reserves, suppresses respiratory metabolism, and restricts the synthesis of proteins required for cell division and elongation. As a result, radicle emergence is delayed or inhibited, leading to reduced germination percentage under higher osmotic stress levels.

In addition to its effects on germination, osmotic stress markedly constrained early seedling growth by limiting cell expansion and biomass accumulation. Reduced water potential decreases cellular turgor pressure, which is critical for cell elongation in both roots and shoots. Prolonged osmotic stress further disrupts hormonal balance, particularly the regulation of growth-promoting hormones such as auxins and gibberellins, thereby suppressing elongation growth and overall seedling vigor. The reduction in seedling length and final weight observed under increasing mannitol concentrations reflects these physiological limitations and indicates compromised growth efficiency under stress conditions.

The more pronounced inhibitory effects recorded at 144 hours compared to earlier observations suggest a cumulative impact of sustained osmotic stress over time. While seedlings may initially tolerate mild water deficit through short-term adaptive responses, prolonged exposure leads to progressive metabolic disruption, reduced reserve utilization, and possible accumulation of stress-induced cellular damage. This time-dependent intensification of stress effects highlights the inability of young seedlings to fully acclimate to continuous osmotic stress during early developmental stages.

Although moth bean is generally recognized as a drought-tolerant crop, the present findings clearly demonstrate that its tolerance is stage-specific and that early developmental phases, particularly germination and initial seedling growth, are highly sensitive to osmotic stress. This sensitivity underscores the importance of ensuring adequate soil moisture during sowing and early establishment to achieve uniform germination and vigorous seedling growth. Moreover, the results emphasize the need for early-stage screening of moth bean genotypes for drought tolerance, as performance during germination and early growth can serve as a reliable indicator of stress resilience under water-limited conditions.

5. Conclusion

The present study clearly demonstrates that mannitol-induced osmotic stress exerts a significant inhibitory effect on germination behavior and early seedling development of moth bean (*Vigna aconitifolia*). Germination percentage, seedling length, and biomass accumulation were progressively reduced with increasing mannitol concentration, indicating a strong concentration-dependent response to osmotic stress. These findings confirm that reduced water potential severely limits the physiological processes required for successful seed germination and subsequent seedling growth.

The results further reveal that the magnitude of growth inhibition intensifies with prolonged exposure to osmotic stress. Seedlings evaluated at 144 hours exhibited more pronounced reductions in growth and biomass compared to earlier stages, highlighting the cumulative nature of stress-induced damage over time. This time-dependent decline suggests that young seedlings possess limited capacity to acclimate to sustained water deficit, resulting in progressive impairment of metabolic activity, cell expansion, and reserve utilization.

Although moth bean is widely regarded as a drought-tolerant crop, the present investigation underscores that its tolerance is highly stage-specific. The early developmental phases, particularly germination and initial seedling establishment, are markedly sensitive to osmotic stress. This vulnerability can critically affect crop stand establishment under rainfed and drought-prone conditions, ultimately influencing productivity.



In conclusion, the study emphasizes the importance of targeting early growth stages in drought tolerance research and crop improvement programs. Screening moth bean genotypes for stress resilience during germination and early seedling development, along with improved moisture management practices at sowing, may play a crucial role in enhancing crop establishment and sustainability in water-limited agroecosystems.

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