

Seed germination response of various cannabis landraces to polyethylene glycol-induced drought stress

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Abstract

This study examined the impact of PEG-induced water stress on seed germination of eight cannabis landraces, namely, 'B1', 'B2', 'B3', 'H1', 'L1', 'L2', 'M1' and 'M2', sourced from four distinct regions of KwaZulu-Natal, South Africa: Bergville (B), Hammersdale (H), Ladysmith (L), and Msinga (M). Drought stress was induced using PEG 8000 (0, -0.2, -0.4, -0.6, -0.8, and -1.0 MPa) in laboratory and greenhouse conditions. Germination percentage (GP), germination rate index (GRI), germination stress tolerance index (GSTI), seedling length, and seed vigour index (SVI) were recorded. In general, there were statistically significant differences ($p < 0.05$) among landraces, PEG treatments, and their interactions for germination characteristics in both greenhouse and laboratory environments. However, the interaction between landraces and PEG treatments did not show statistical differences ($p > 0.05$) in germination percentage under greenhouse conditions. The germination characteristics were

reduced under PEG treatments relative to the control. Under laboratory conditions, germination declined progressively with increasing PEG concentration. Complete inhibition of GP occurred at -1.0 MPa in landraces ‘M1’ and ‘L1’. However, at this stress level, ‘L2’ had the highest GP of 58.67% and GRI of 1.78 seeds/day, while ‘B1’ exhibited the highest GSTI at 18.41%. In corresponding greenhouse experiment at -1.0 MPa, ‘H1’ maintained the highest GP of 92.00%, while ‘B3’ achieved the highest GRI (5.28 seeds/day), GSTI (100.99%), seedling length (167.7 mm), and SVI (1.114). This study identified ‘L2’, ‘B1’, ‘H1’, and ‘B3’ as promising landraces for cultivation in water-limited environments and for use in breeding programs.

Keywords: Cannabis landrace, polyethylene glycol, germination recovery, drought stress

1. Introduction

Climate change directly and significantly impacts agricultural production as it influences various climate variables, including temperature, rainfall patterns, growing seasons, humidity, water availability, crop genotype selection, and soil health (Maponya and Mpandeli, 2012). Changes in these factors can disrupt traditional agricultural practices and have far-reaching consequences for crop yields, thus threatening sustainability, food security, and livelihoods of the farmers who primarily depend on rain for crop production (Schreiner *et al.*, 2018; Myeni *et al.*, 2019). Of these changes, drought and water stress have the most significant impact on crop production. Water stress interferes with “plant cellular functions, including protein synthesis, nitrogen assimilation, and cell membrane activity” (Zia *et al.*, 2021). Water-stressed plants typically exhibit stunted growth, lower turgor pressure, and limited transpiration, prompting growers to increasingly depend on irrigation (Materechera, 2011; Jordán and Speelman, 2020; Zia *et al.*, 2021).

Cannabis sativa is a versatile crop with numerous applications, contributing to various products and industries such as “agriculture, textiles, biocomposites, paper production, automotive

manufacturing, construction, biofuels, functional foods, oils, cosmetics, and personal care products” (Salentijn *et al.*, 2015; Chaohua *et al.*, 2016). Cannabis has therapeutic and pharmaceutical properties owing to its abundance of phenolic compounds, terpenoids, and cannabinoids (Grulichova *et al.*, 2017; Żuk-Gołaszewska and Gołaszewski, 2018). The cannabis crop is highly adaptable and grows in various climatic and agronomical conditions (Decorte *et al.*, 2011). It thrives in loose, well-drained loamy soil that is highly fertile, rich in organic matter, and receives ample sunlight, warmth, and moisture (Clarke and Merlin, 2016; Sera *et al.*, 2017). Cannabis growth requires good soil moisture and adequate rainfall, especially during the first six weeks of sowing (Sera *et al.*, 2017). However, with reduced precipitation due to climate change, the required water during seed germination and seedling growth may not be met, ultimately hindering successful seed emergence (Zia *et al.*, 2021).

“It is well understood that seed germination is the initial, most critical, and vulnerable phase in the life cycle of plants, and the successful establishment of seedlings largely depends on germination” (Gorai and Neffati, 2007; Petrovic *et al.*, 2016). Water availability is crucial for seed germination for various processes, such as “imbibition, enzyme activation, embryo stimulation, reserve mobilization, and plumule emergence and elongation” (Wijewardana *et al.*, 2018; Wolny *et al.*, 2018; López *et al.*, 2021; Langa *et al.*, 2024a). Insufficient water availability reduces both the percentage and rate of germination, ultimately leading to poor seed germination (Wijewardana *et al.*, 2018; Rahmah *et al.*, 2020; Langa *et al.*, 2024a). Additionally, it results in increased developmental mortality and reduced crop establishment success (Tounekti *et al.*, 2020; Langa *et al.*, 2024a).

Research studies on drought response and tolerance during germination are very limited in cannabis crops, particularly in South African landraces, due to the historical ban of the crop from usage for research and industrial applications for the past centuries. Such research is now critical following the recent legalization of cannabis cultivation and the rapidly increasing

demand for hemp products (Schlottenhofer and Yuan, 2017; Citti *et al.*, 2018). Understanding how seeds respond to water-deficit conditions during germination is essential for identifying drought-tolerant varieties that can maintain productivity under climate stress (Petrovic *et al.*, 2016). PEG is a natural, water-soluble, and nonionic polymer commonly used to simulate water-stress conditions as it reduces the water potential in plants by causing osmotic effects (Petrovic *et al.*, 2016; Ahmad *et al.*, 2020). As an inert osmotic agent with molecules too large to enter the seed, PEG induces stress without causing toxicity, making it suitable for studying drought responses in plants (Petrovic *et al.*, 2016; Shivakrishna *et al.*, 2018).

PEG has been previously used to evaluate the impact of drought stress on cannabis, including studies by Li *et al.* (2018), Blandinières *et al.* (2021), and Du *et al.* (2022). However, Li *et al.* (2018) and Du *et al.* (2022) studied the effect of combining pre-treatments and drought stress under a single concentration of 20% PEG. Blandinières *et al.* (2021) also used a similar concentration, and these studies were conducted in industrial hemp, whereas different varieties might have a different response to different PEG concentrations. Thus, the response of different cannabis varieties to different PEG concentrations is still limited. Certain seeds can retain their viability even after prolonged exposure to drought stress and germinate once the abiotic stress is alleviated (Ahmadloo *et al.*, 2011; Vicente *et al.*, 2020). However, such information is limited in cannabis. Therefore, the objective of the current study was to investigate the response of different cannabis landraces to PEG-induced drought stress under laboratory and greenhouse conditions.

2. Methods and materials

2.1. Sampling of cannabis landraces

Seeds from eight locally adapted cannabis landraces were collected from small-scale farmers across four KwaZulu-Natal regions, each assigned standardized codes (Figure 1): Bergville

Natal ('B2'), Ugwayi wesiZulu ('B1') and Iswazi ('B3'), Hammersdale Durban Poison ('H1'), Ladysmith Ugwayi wesiZulu ('L1') and Iswazi ('L2'), and Msinga Ugwayi wesiZulu ('M1') and Iswazi ('M2'). Although analyzing seeds grown under the same conditions with known morphological characteristics and agronomic performance was preferred, planting for grain or seed production was not feasible due to funding limitations. Consequently, the study used the collected seeds in their existing state.

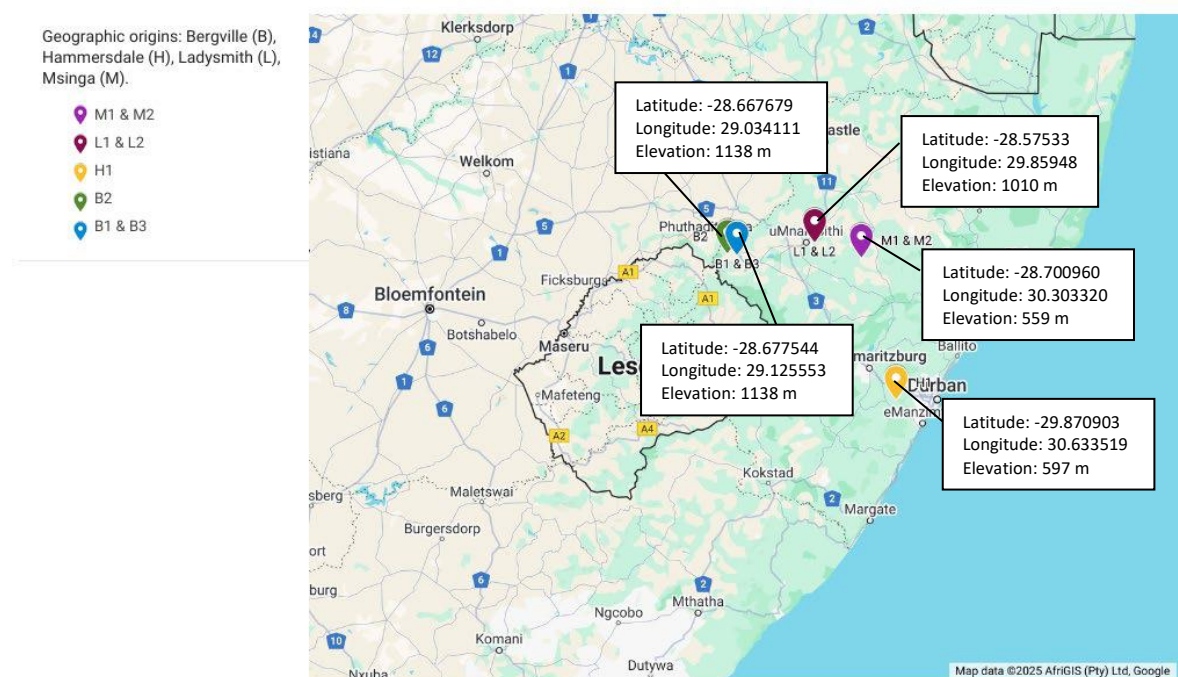


Figure 1: Eight cannabis landraces from KwaZulu-Natal smallholders, coded by origin: Bergville ('B1', 'B2', 'B3'), Hammersdale ('H1'), Ladysmith ('L1', 'L2'), and Msinga ('M1', 'M2').

Following collection, seed samples received no climate control and were preserved under naturally varying ambient storage conditions in the Horticultural Sciences Laboratory at the University of KwaZulu-Natal, Pietermaritzburg (GPS coordinates: -29.636311, 30.409060).

Before conducting germination tests, seeds were visually sorted by colour as an indicator of maturity: dark-coloured seeds were selected for the experiment as they represented fully mature specimens, while greenish seeds (indicating immaturity) were excluded (Miraji *et al.*, 2021). Any damaged or visibly defective seeds were also eliminated from the experimental batch.

2.2. Experimental design

The study evaluated drought responses in cannabis landraces through laboratory (Petri dishes) and greenhouse (polystyrene trays) trials, using a factorial completely randomized design with three replicates per treatment (Langa *et al.*, 2024b). The two experimental factors were eight landraces and six polyethylene glycol (PEG) treatments. Drought stress was simulated using polyethylene glycol (PEG) BioUltra 8000 (Sigma-Aldrich, Merck KGaA, Darmstadt, Germany) following the method of Midaoui *et al.* (2003) with some modifications. While Midaoui *et al.* (2003) used PEG 6000 at concentrations of 0, -0.6, and -1.0 MPa, this study prepared PEG 8000 solutions by dissolving the polymer in distilled water at 25 ± 2 °C. The concentrations were set at -0.2, -0.4, -0.6, -0.8, and -1.0 MPa, with intervals of -0.2 MPa, while distilled water served as the control treatment. The amount of PEG required for each concentration was calculated using Equation (Eq.) 1, as described by Michel (1983).

$$\text{PEG} = \frac{4 - (5.16\phi T - 560\phi + 16)^{0.6}}{2.58T - 280} \quad (1)$$

Where ϕ represents water potential and T represents Celsius temperature.

2.3. Seed viability test

Viability testing was performed using 2,3,5-triphenyltetrazolium chloride (TTC) (Sigma-Aldrich, Merck KGaA, Germany), following methods described by Mandizvo and Odindo (2019). TTC is a quick, effective water-soluble dye that changes colour in living seed tissues due to dehydrogenase enzyme activity (Paiva *et al.*, 2017; Virgens *et al.*, 2019; Langa *et al.*,

2025). The seeds were immersed in distilled water for 18 hours at 25°C, then cut through the embryo and soaked in 1% TTC solution for 12 hours at 25 °C. The test was repeated three times with 30 seeds per replicate. Seeds were classified as viable if their embryos showed a uniform pink colour and non-viable if white. Seed viability was expressed as the percentage of viable seeds.

2.4. Germination test

The germination test was conducted following methods by Langa *et al.* (2024b). Briefly, cannabis seeds underwent 24-hour hydration in distilled water before being subjected to germination trials to initiate germination. For each landrace, 25 seeds were placed on moistened cotton pads in 90 mm Petri dishes (laboratory conditions), and another 25 were sown in polystyrene trays (greenhouse) filled with lawn dressing compost (Duzi Turf, Pietermaritzburg). This compost is a fine, humus-rich (85%), weed-free medium made from processed organic waste and sewage sludge, containing 15% particulate matter sized 2-8 mm. The Petri dishes were transferred to a controlled-environment chamber (Micro-Clima Arabidopsis Chamber, Snijders) set at 30/25 °C with a 16/8-hour light/dark cycle, while the seedling trays were kept under greenhouse conditions. Both setups were replicated three times and monitored daily, over 7 days in the laboratory and 14 days in the greenhouse. Moisture was maintained through regular irrigation with tap water. Germination was recorded when the radicle reached 2 mm or longer, and germination percentage (GP) was calculated using formula (Eq. 2) by Khalaki *et al.* (2019).

$$\text{Germination percentage (GP)} = \frac{\text{Number of germinated seeds}}{\text{Total number of seeds}} \times 100 \quad (2)$$

2.5. Germination recovery

Seeds that did not germinate during the laboratory PEG treatments were rinsed and placed in Petri dishes with distilled water for an additional 3 days. This procedure evaluated seed

viability post-stress by quantifying successful germination after stress alleviation. The number of recovered seeds was added to those that germinated under PEG treatments. Germination recovery percentage was determined using Eq. 3 (Chen *et al.*, 2022).

$$\text{Germination recovery percentage (GRP)} = \frac{a+b}{c} \times 100 \quad (3)$$

Where a represents the number of seeds that germinated following stress relief, b denotes the number of seeds that germinated during the water stress treatment, and c is the total number of seeds tested.

2.6. Germination rate index

Following the recovery period, total germination counts were recorded, and the germination rate index (GRI). The germination rate index was calculated using Eq. 4 (Awasthi *et al.*, 2016).

$$\text{GRI} = \frac{\text{Number of germinated seeds}}{\text{Day of first count}} + \dots + \frac{\text{Number of germinated seeds}}{\text{Day of final count}} \quad (4)$$

2.7. Germination stress tolerance index

The total number of germinated seeds was recorded and subsequently used to calculate the germination stress tolerance in cannabis landraces under water stress conditions. The germination stress index was calculated using the Promptness Index (PI), which was calculated using Eq. 5 (Giancarla *et al.*, 2012).

$$\text{PI} = \text{nd2} (1.0) + \text{nd4} (0.8) + \text{nd6} (0.6) + \text{nd8} (0.4) + \text{nd10} (0.2) \quad (5)$$

Where nd2 , nd4 , nd6 , nd8 , and nd10 represent the number of germinated seeds in 2nd, 4th, 6th, 8th, and 10th day, respectively.

After that, the Germination Stress Tolerance Index (GSTI) was calculated using Eq. 6 by Giancarla *et al.* (2012).

$$\text{GSTI (\%)} = \frac{PIS}{PINS} \times 100 \quad (6)$$

Where *PIS* is the promptness index under water stress conditions, and *PINS* is *PI* under control.

2.8. Seedling development assessment

Post-germination, the length of every seedling was recorded in millimeters (mm) using a ruler, with mean values calculated per landrace and treatment group. The Seedling Vigour Index (SVI) was then determined by combining the final germination percentage with the average seedling length, following Eq. 7 from Awasthi *et al.* (2016).

$$\text{SVI} = \frac{\text{Final germinated percentage} \times \text{Seedling length}}{100} \quad (7)$$

2.9. Data analyses

Statistical analysis was conducted using GenStat® version 20.1 (VSN International, Hemel Hempstead, UK, 2020), applying a significance threshold of 5%. Differences among means were determined using Tukey's multiple comparison test. Furthermore, multivariate statistical analysis, including correlation and Agglomerative Hierarchical Clustering (AHC), were performed using the R Statistical Computing Environment (version 4.4.0) on the RStudio platform (Posit Software, PBC, 250 Northern Ave Suite 420, Boston, MA 02210).

3. Results

3.1. Seed viability

Significant differences ($p < 0.001$) in seed viability were observed among the cannabis landraces, as determined by the TTC test. Landrace 'B1' recorded the highest viability at 95.67%, while 'L1' had the lowest at 70.00% (Figure 2). Although 'H1' and 'M1' both showed a viability of 76.67%, the differences between these landraces and 'L1' and 'M2' were not statistically significant.

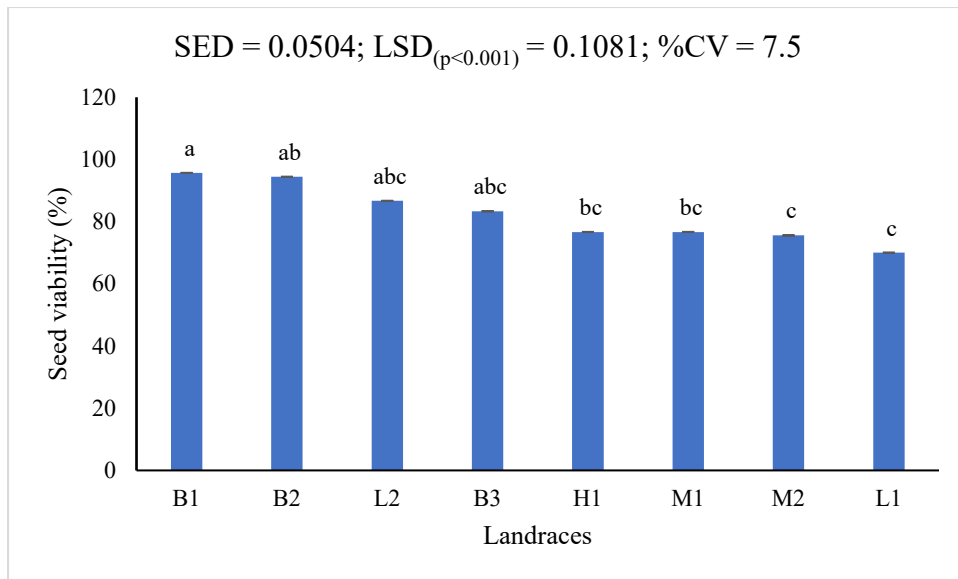


Figure 2: Seed viability of cannabis landraces under varying PEG concentrations ($p < 0.001$; $n = 3$ replicates). Columns with identical letters indicate no significant difference; while differing letters signify statistically significant differences based on Tukey's test. SED refers to the standard error of difference; LSD (5%) denotes the least significant difference at the 5% significance level; %CV represents the coefficient of variation.

3.2. Laboratory germination performance

Statistical analysis revealed highly significant effects ($p < 0.001$) of landrace, PEG treatment, and their interaction on all measured germination parameters under laboratory conditions.

3.2.1. Germination

Germination of all cannabis landraces declined with increasing PEG concentrations (Figure 3). Overall, 'L2' showed the highest germination, while 'L1' had the lowest, with complete inhibition (0% germination) under PEG -0.6, -0.8, and -1.0 MPa. 'H1' had the lowest germination under control conditions and was also completely inhibited under PEG -1.0 MPa, similar to 'B3' and 'M2'. Similar to 'L1', the germination of 'M1' was completely inhibited

under PEG -0.6, -0.8 and -1.0 MPa. Additionally, ‘B2’ exhibited complete inhibition under PEG -0.8 and -1.0 MPa.

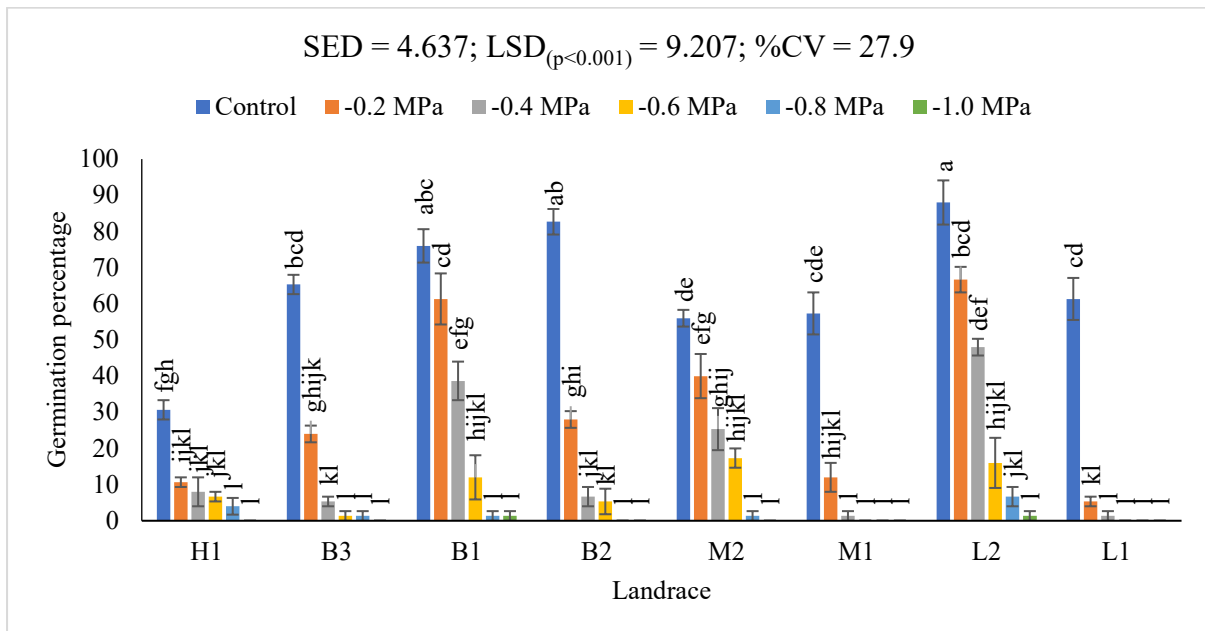


Figure 3: Germination of various cannabis landraces in response to different PEG concentrations under laboratory conditions ($p < 0.001$; $n = 3$ replicates). Columns with identical letters indicate no significant difference; while differing letters signify statistically significant differences based on Tukey’s test. SED refers to the standard error of difference; LSD (5%) denotes the least significant difference at the 5% significance level; %CV represents the coefficient of variation.

3.2.2. Germination recovery

Germination after recovery generally declined with increasing PEG concentration (Figure 4). However, notable variations were observed in ‘H1’, ‘M2’, and ‘L2’. ‘H1’ showed increased germination under PEG -0.6, -0.8, and -1.0 MPa (26.67%, 34.67%, and 18.67%, respectively) compared to 16.00% at PEG -0.4 MPa. ‘M2’ had higher germination at PEG -0.8 MPa (41.33%) than at PEG -1.0 MPa (36.00%). Similarly, ‘L2’ showed improved germination under PEG -0.8 and -1.0 MPa (42.67% and 49.33%) compared to 25.33% at PEG -0.6 MPa. In

contrast, ‘M1’ and ‘L1’ showed no recovery, while ‘L2’ had the highest overall germination and ‘L1’ the lowest.

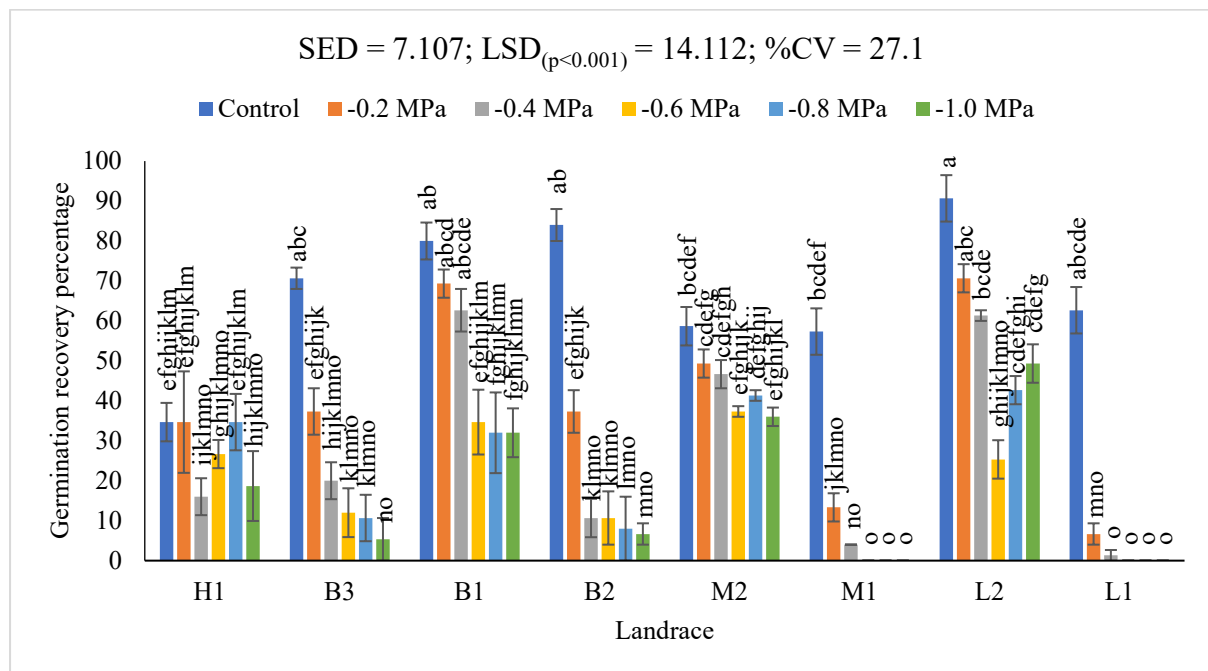


Figure 4: Germination recovery of various cannabis landraces subjected to different PEG concentrations under laboratory conditions ($p < 0.001$; $n = 3$ replicates). Columns with identical letters indicate no significant difference; while differing letters signify statistically significant differences based on Tukey’s test. SED refers to the standard error of difference; LSD (5%) denotes the least significant difference at the 5% significance level; %CV represents the coefficient of variation.

3.2.3. Germination rate index

A general reduction in the germination rate index (GRI) was observed as PEG concentrations increased (Figure 5). However, notable variations were observed in the interaction between landraces and PEG treatments, particularly in ‘H1’ and ‘M2’. ‘H1’ showed higher GRI at PEG -0.6 and -0.8 MPa (1.083 and 1.442 seeds/day) compared to 0.868 seeds/day at PEG -0.4 MPa. Similarly, ‘M2’ had increased GRI under PEG -0.8 and -1.0 MPa (1.396 and 1.479 seeds/day) compared to 1.339 at PEG -0.6 MPa. ‘B1’ also showed a slight GRI increase at PEG -1.0 MPa

(0.971 seeds/day) compared to PEG -0.8 MPa (0.922 seeds/day). Overall, ‘L2’ had the highest GRI, while ‘L1’ had the lowest across treatments.

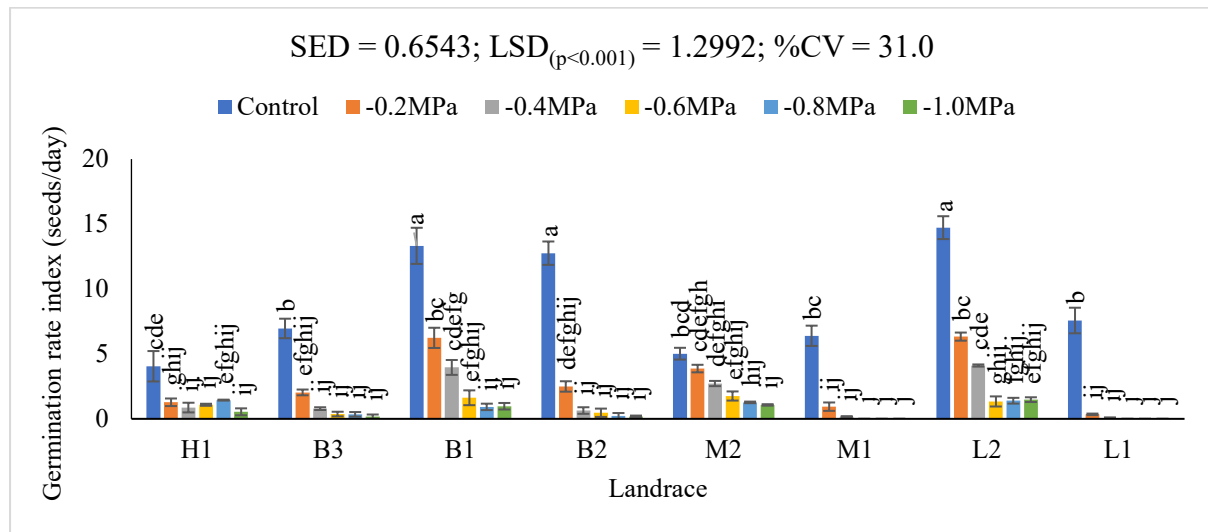


Figure 5: Germination rate index of various cannabis landraces subjected to different PEG concentrations under laboratory conditions ($p < 0.001$; $n = 3$ replicates). Columns with identical letters indicate no significant difference; while differing letters signify statistically significant differences based on Tukey’s test. SED refers to the standard error of difference; LSD (5%) denotes the least significant difference at the 5% significance level; %CV represents the coefficient of variation.

3.2.4. Germination stress tolerance index

The germination stress tolerance index (GSTI) generally declined with increasing PEG concentrations under laboratory conditions, particularly in landraces ‘B3’, ‘B2’, ‘M1’, and ‘L1’ (Figure 6). However, notable variations in GSTI responses were observed among certain landraces, including ‘H1’, ‘B1’, ‘M2’, and ‘L2’, which recorded the highest values across different PEG levels. For instance, ‘H1’ showed a sharp increase in GSTI at PEG -0.6 and -0.8 MPa (45.75% and 102.09%, respectively) compared to 17.60% at PEG -0.4 MPa, with the highest overall GSTI recorded at PEG -0.8 MPa. Similarly, ‘B1’ demonstrated higher GSTI at PEG -1.0 MPa (18.41%) compared to under PEG -0.8 MPa (14.86%). ‘L2’ showed improved

GSTI at PEG -0.8 and -1.0 MPa (28.83% and 21.52%, respectively) compared to 18.43% at PEG -0.6 MPa. ‘M2’ also showed a slightly higher GSTI under PEG -0.4 MPa (85.98%) compared to PEG -0.2 MPa (85.02%) and a higher value at PEG -0.8 MPa (45.44%) than at PEG -0.6 MPa (41.11%). In contrast, ‘L1’ and ‘M1’ consistently exhibited the lowest GSTI across all treatments. Overall, ‘M2’, followed by ‘B1’, displayed the greatest tolerance to PEG-induced drought stress.

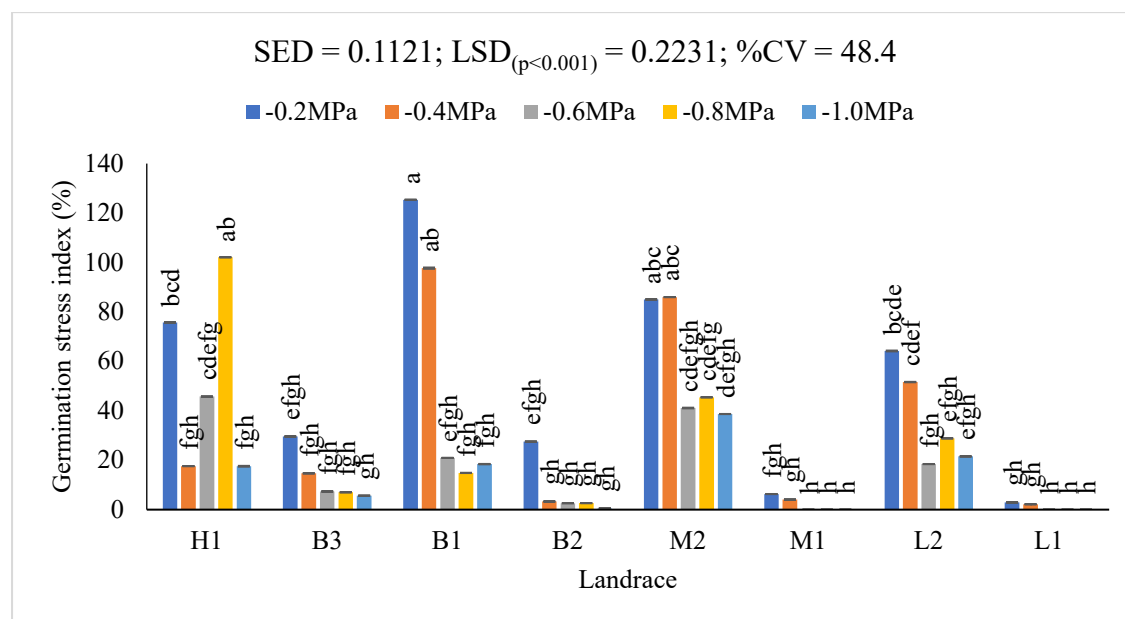


Figure 6: Germination stress index of various cannabis landraces subjected to different PEG concentrations under laboratory conditions ($p < 0.001$; $n = 3$ replicates). Columns with identical letters indicate no significant difference; while differing letters signify statistically significant differences based on Tukey’s test. SED refers to the standard error of difference; LSD (5%) denotes the least significant difference at the 5% significance level; %CV represents the coefficient of variation.

3.2.5. Multivariate analysis

3.2.5.1. Correlation matrix

Pearson’s correlation (Figure 7) revealed a strong and significant positive relationship among all germination parameters of cannabis landraces ($p < 0.001$), suggesting that as the one variable

decreased due to the effect of PEG the other variables also decreased. However, GSTI showed a slightly weaker association with the other parameters.

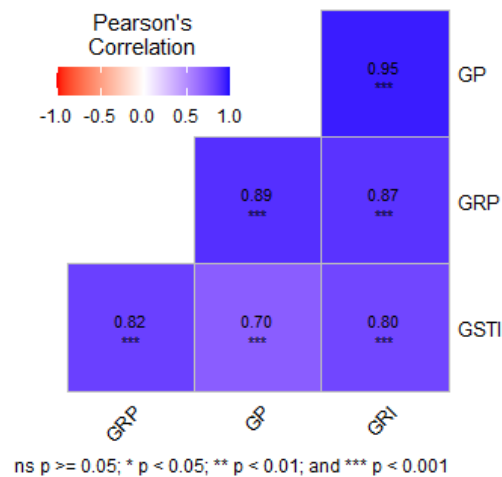


Figure 7: Pearson's correlation showing a relationship between germination parameters measured in cannabis landraces subjected to PEG treatments under laboratory conditions

3.2.5.2. Hierarchal Cluster Analysis

The dendrogram grouped the landraces into three main clusters (Figure 8). The first cluster consisted of 'B1' and 'L2', which were highly similar but distinct from the other accessions. The second cluster comprised 'L1', 'M1', 'B2', and 'B3', forming two sub-groups (L1'-'M1' and 'B2'-'B3') that later merged at a higher dissimilarity level. The third cluster included 'H1' and 'M2', which showed moderate similarity and only joined the main structure at a relatively high dissimilarity.

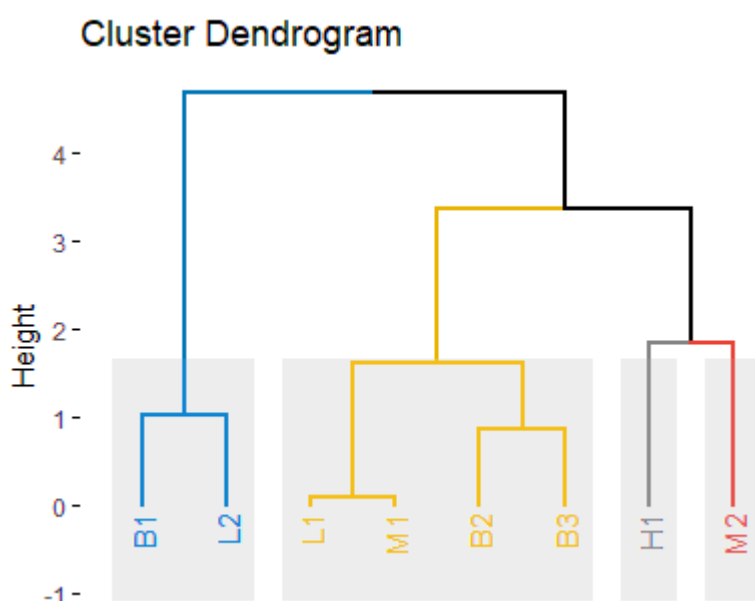


Figure 8: Hierarchical cluster of cannabis landraces based on seed germination, germination recovery and rate index and germination stress tolerance index

3.3. Greenhouse germination and seedling development performance

The landraces, PEG treatment, and their interaction demonstrated significant differences ($p < 0.05$) in germination rate index, seedling length, seed vigour, and germination stress index. The landraces and PEG treatment also showed significant differences ($p < 0.05$) in germination percentage. Meanwhile, the interaction between landrace and PEG treatments revealed no significant difference ($p > 0.05$) in germination percentage.

3.3.1. Germination

Germination generally declined with increasing PEG concentrations across all cannabis landraces (Figure 9). However, 'B1' showed a slight increase under PEG -0.6 MPa (78.67%) compared to PEG -0.4 MPa (77.33%). Notably, 'M1' and 'L2' showed no significant difference in germination under PEG -0.2 MPa compared to the control, with 'M1' maintaining 86.67% and 'L2' 77.33% in both treatments. Overall, 'H1' exhibited the highest germination, followed by 'B3', while 'B2' and 'L2' showed the lowest.

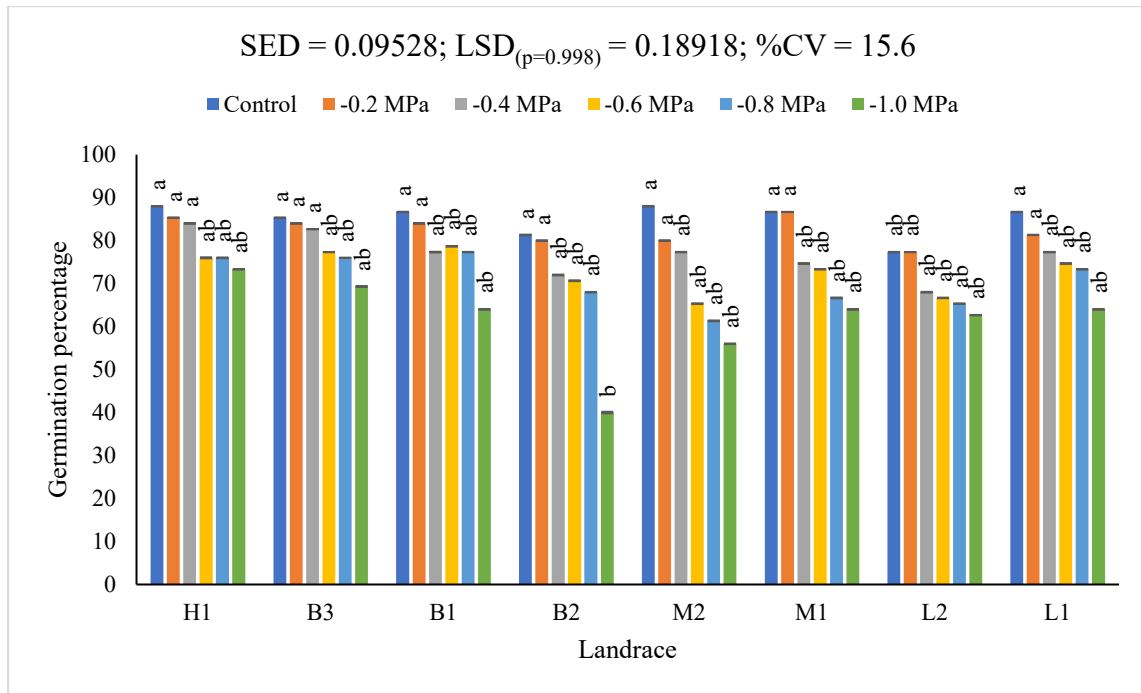


Figure 9: Germination of various cannabis landraces subjected to different PEG concentrations under greenhouse conditions ($p=0.998$; $n = 3$ replicates). Columns with identical letters indicate no significant difference; while differing letters signify statistically significant differences based on Tukey's test. SED refers to the standard error of difference; LSD (5%) denotes the least significant difference at the 5% significance level; %CV represents the coefficient of variation.

3.3.2. Germination rate index

Variation in the germination rate index was observed among the cannabis landraces in response to different PEG concentrations (Figure 10), with the highest rates generally observed under control. However, 'H1', 'M1', and 'M2' exhibited increased germination rate indices under certain PEG treatments compared to the control. Specifically, 'H1' showed the highest germination rate index of 3.365 and 3.621 seeds/day under PEG -0.2 and -0.4 MPa, respectively, while the control recorded 3.285 seeds/day. 'M1' exhibited the highest germination rate index of 5.129 seeds/day under PEG -0.2 MPa, with control recorded 4.660

seeds/day. ‘M2’ demonstrated the highest germination rate index of 6.443 seeds/day under PEG -0.2 MPa, exceeding the 4.423 seeds/day recorded under control. Overall, ‘B3’ and ‘B1’ exhibited the highest germination rate indices under PEG treatments, whereas ‘H1’ and ‘M2’ generally showed the lowest.

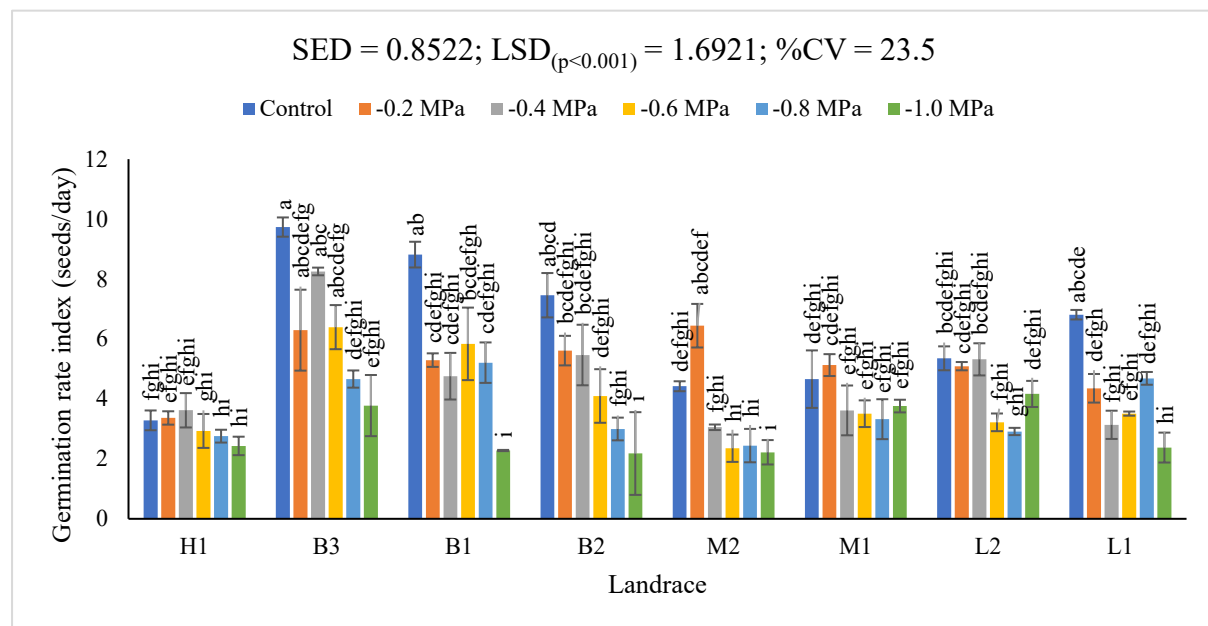


Figure 10: Germination rate index of various cannabis landraces subjected to different PEG concentrations under greenhouse conditions ($p < 0.001$; $n = 3$ replicates). Columns with identical letters indicate no significant difference; while differing letters signify statistically significant differences based on Tukey’s test. SED refers to the standard error of difference; LSD (5%) denotes the least significant difference at the 5% significance level; %CV represents the coefficient of variation.

3.3.3. Germination stress tolerance index

The findings of the study indicated that germination stress tolerance index differed across cannabis landraces and PEG treatments under greenhouse conditions (Figure 11). Overall, the index decreased with increasing PEG concentration, reaching its lowest values at PEG -1.0 MPa for most landraces, except for ‘H1’ and ‘M2’. Notably, ‘H1’ exhibited the highest

germination stress tolerance index of 100.99% at PEG -1.0 MPa, with its lowest value of 90.36% recorded at PEG -0.4 MPa. Similarly, ‘M2’ showed a higher index of 83.09% under PEG -1.0 MPa compared to 79.96% at PEG -0.8 MPa. Among the landraces, ‘B3’, ‘B1’, and ‘B2’ displayed the highest indices at PEG -0.4 MPa, with values of 222.17%, 89.16%, and 64.21%, respectively. Overall, ‘B3’ consistently showed a higher germination stress tolerance index across PEG treatments, while ‘B2’ and ‘L1’ generally exhibited lower tolerance levels.

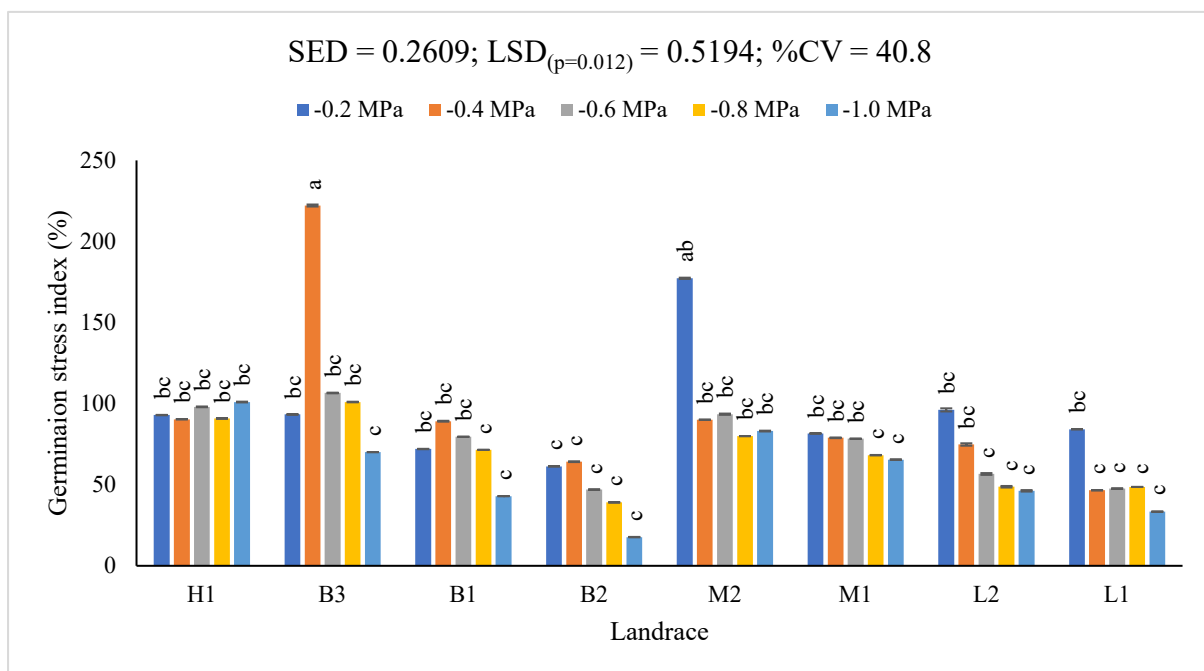


Figure 11: Germination stress index of various cannabis landraces subjected to different PEG concentrations under greenhouse conditions ($p=0.012$; $n = 3$ replicates). Columns with identical letters indicate no significant difference; while differing letters signify statistically significant differences based on Tukey’s test. SED refers to the standard error of difference; LSD (5%) denotes the least significant difference at the 5% significance level; %CV represents the coefficient of variation.

3.3.4. Seedling length

Seedling length differed across cannabis landraces and varied in response to different PEG concentrations (Figure 12). In general, seedling length was highest under the control treatment

and lowest under PEG -1.0 MPa. Notably, ‘M2’ and ‘M1’ recorded the longest seedling lengths of 167.7 mm under PEG -1.0 MPa and 155.3 mm under PEG -0.6 MPa, respectively. Conversely, ‘M2’ and ‘M1’ also exhibited the shortest seedling lengths of 107.0 mm and 115.0 mm under PEG -0.2 MPa and -0.4 MPa, respectively. Similarly, ‘H1’ and ‘B3’ recorded the lowest lengths of 101.1 mm and 136.1 mm under PEG -0.8 MPa. For ‘B1’, ‘B2’, ‘L2’, and ‘L1’, the shortest seedling lengths were observed under PEG -1.0 MPa at 113.5 mm, 82.8 mm, 129.1 mm, and 103.5 mm, respectively. Across treatments, seedling length tended to be higher under PEG -0.6 MPa compared to PEG -0.2 and -0.4 MPa. Among the landraces, ‘B3’ generally exhibited the greatest seedling length, while ‘H1’ had the lowest under PEG-induced drought stress conditions.

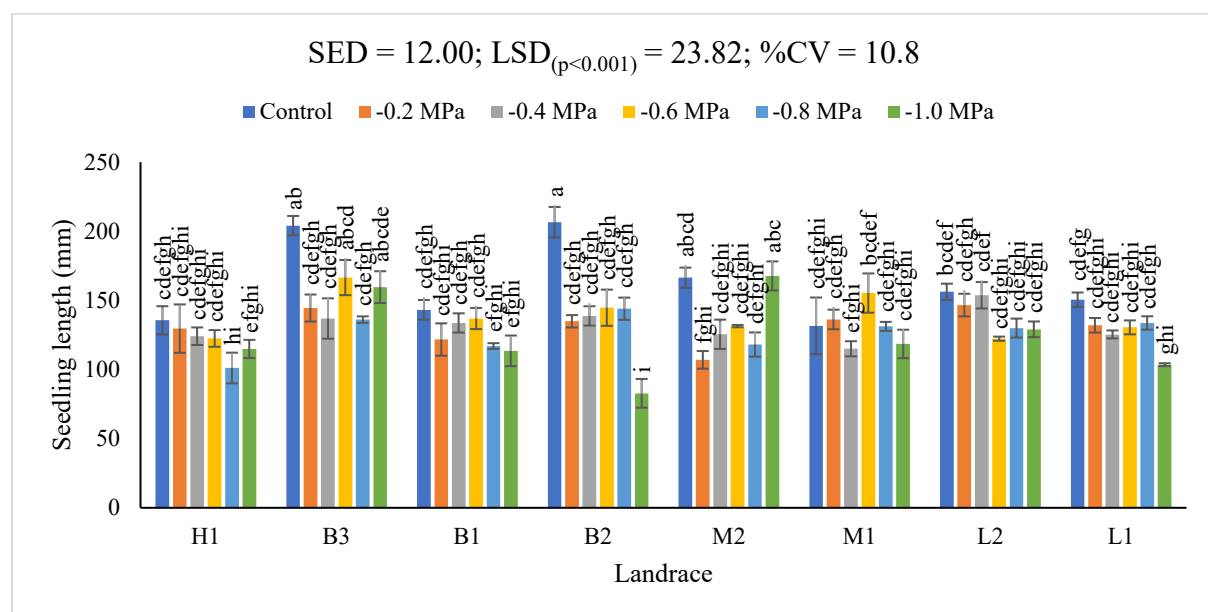


Figure 12: Seedling length of various cannabis landraces subjected to different PEG concentrations under greenhouse conditions ($p < 0.001$; $n = 3$ replicates). Columns with identical letters indicate no significant difference; while differing letters signify statistically significant differences based on Tukey’s test. SED refers to the standard error of difference; LSD (5%) denotes the least significant difference at the 5% significance level; %CV represents the coefficient of variation.

3.3.5. Seed vigour index

The seed vigour index showed variation across different cannabis landraces and PEG concentration levels (Figure 13). Across all genotypes, the seed vigour index was significantly reduced under PEG-induced drought stress compared to control conditions, except for ‘M1’, which recorded its highest index of 1.184 under PEG -0.2 MPa. Notably, ‘H1’, ‘B3’, and ‘M2’ exhibited their lowest seed vigour indices under PEG -0.8 MPa (0.759, 1.035, and 0.738, respectively), which were lower than those recorded under PEG -1.0 MPa (0.841, 1.114, and 0.929, respectively). Meanwhile, the other landraces had the lowest seed vigour under PEG -1.0 MPa. Additionally, ‘B3’, ‘B1’, ‘M1’, and ‘L1’ recorded higher seed vigour indices under PEG -0.6 MPa (1.297, 1.067, 1.124, and 0.974, respectively) compared to those under PEG -0.4 MPa (1.127, 1.026, 0.863, and 0.967, respectively). In both ‘B3’ and ‘B1’, seed vigour indices under PEG -0.6 MPa were also higher compared to those recorded under PEG -0.2 MPa (1.214 and 1.018, respectively). Overall, ‘B3’ and ‘M2’ generally exhibited the highest and lowest seed vigour indices, respectively, across the PEG treatments.

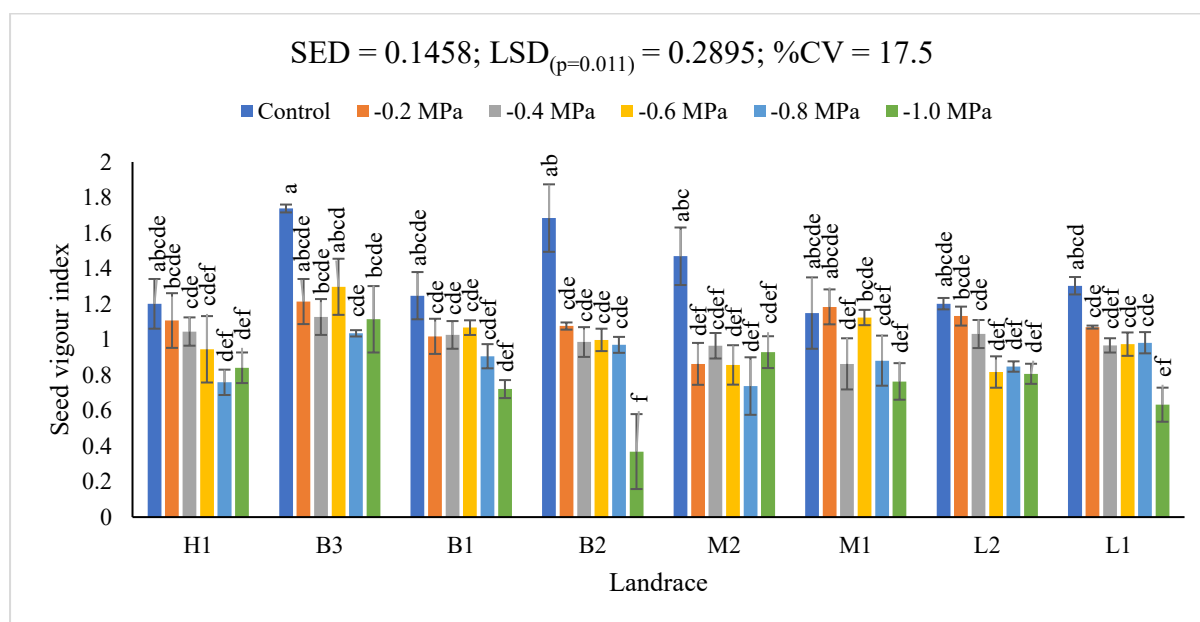


Figure 13: Seed vigour index of various cannabis landraces subjected to different PEG concentrations under greenhouse conditions ($p=0.011$; $n = 3$ replicates). Columns with

identical letters indicate no significant difference; while differing letters signify statistically significant differences based on Tukey's test. SED refers to the standard error of difference; LSD (5%) denotes the least significant difference at the 5% significance level; %CV represents the coefficient of variation.

3.3.6. Multivariate analysis

3.3.6.1. Correlation matrix

Pearson's correlation (Figure 14) revealed a strong and significant positive relationship among most germination parameters of cannabis landraces ($p < 0.001$). The seedling vigor index (SVI) showed very strong correlations with seedling length (SL; $r = 0.87$) and germination percentage (GP; $r = 0.74$), as well as a strong correlation with the germination rate index (GRI; $r = 0.72$). Moderate positive correlations were observed between GP and both SL ($r = 0.59$) and GRI ($r = 0.58$), while GP was also moderately correlated with the germination stress tolerance index (GSTI; $r = 0.50$). In contrast, GSTI displayed a weak correlation with SVI ($r = 0.40$, $p < 0.05$) and nonsignificant correlation with SL ($r = 0.13$, ns). These results suggest that germination percentage, seedling length, and vigor were closely interrelated, whereas GSTI demonstrated a more independent aspect of seed performance under stress.

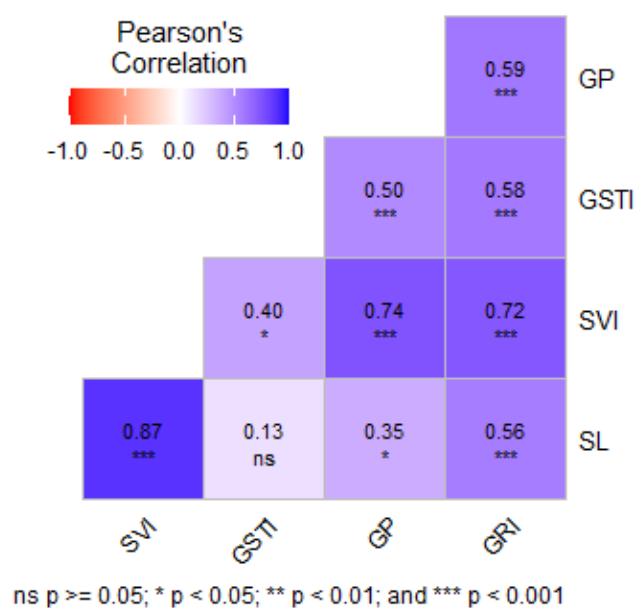


Figure 14: Pearson's correlation showing a relationship between germination parameters measured in cannabis landraces subjected to PEG treatments under greenhouse conditions

3.3.6.2. Hierarchal Cluster Analysis

Hierarchical clustering revealed three primary groups and one outlier (Figure 15). The first cluster contained 'H1', 'B1', 'L1', and 'M1', with 'L1' and 'M1' being the most closely related. The second cluster comprised the closely related 'B2' and 'L2'. The third cluster contained only the distinct 'M2'. In contrast, 'B3' was identified as a clear outlier, demonstrating the greatest genetic divergence from all other accessions.

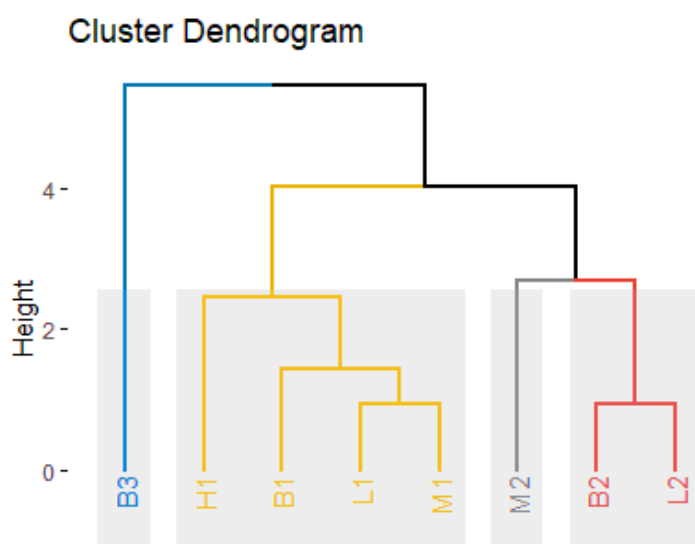


Figure 15: Hierarchical cluster of cannabis landraces based on seed germination, germination rate index, germination stress tolerance index, seedling length and seed vigour index

4. Discussion

4.1. Germination

The germination percentage in cannabis landraces generally decreased with the increasing PEG concentration under both laboratory and greenhouse conditions, with sharper reductions observed under laboratory conditions. The lower germination in the laboratory compared to the greenhouse can be attributed to the precisely controlled conditions, which maintained constant temperature and relative humidity, thereby increasing the effects of reduced water potential. The decline in germination under PEG treatments is consistent with the osmotic stress imposed by PEG 8000, which lowers the ability of seeds to imbibe sufficient water for germination (Zia *et al.*, 2021). As a result, the metabolic processes of the seeds are disrupted and may cease altogether (Violita and Azhari, 2021). Such disruption includes degradation and inactivation of essential hydrolytic enzymes that convert endosperm starch into simple sugars necessary for germination, ultimately reducing germination percentage (Vijay *et al.*, 2018; Bukhari *et al.*, 2021). These findings corroborate earlier reports that increasing PEG concentrations reduced

germination percentage, germination rate index, and mean germination time in maize (Queiroz *et al.*, 2019), and that water deficit stress reduced dry matter production in hemp (Babaei and Ajdanian, 2020). Collectively, limited water availability restricts plant growth attributes from germination through to harvest.

Although germination was reduced under PEG-induced stress, recovery was still observed when seeds were transferred to distilled water, indicating that viability was maintained under high-stress conditions. However, variation existed among landraces, with control treatments consistently showing the highest germination. The failure of ‘M1’ and ‘L1’ to germinate under severe stress was associated with their limited physiological tolerance to drought. This is consistent with the observation that drought stress can damage genes and inhibit gibberellin synthesis, thereby reducing α -amylase activity and impeding starch mobilization required for germination (Balouch *et al.*, 2023). Conversely, recovery in other landraces under PEG stress was linked to their ability to remain viable and germinate once stress was alleviated (Vicente *et al.*, 2020). Although genetic studies on these landraces are limited, differences in recovery ability likely reflect genetic influences on traits such as moisture uptake efficiency. Landraces such as ‘M1’ and ‘L2’ demonstrated adaptability under mild stress (-0.2 MPa), with germination percentages similar to the control, suggesting that lower PEG concentrations did not sufficiently disrupt seed metabolism. These results agree with findings in sorghum, where PEG -0.2 MPa did not significantly limit germination compared to the control (Queiroz *et al.*, 2019).

4.2. Germination rate index

Similarly, the germination rate index decreased with higher PEG concentrations, consistent with the understanding that seeds require additional time to adjust their osmotic potential under stress (Queiroz *et al.*, 2019; Zia *et al.*, 2021). PEG disrupts membranes and cellular

components, requiring seeds to restore antioxidant activity before normal metabolism resumes (Balouch *et al.*, 2023). Comparable reductions in GRI under PEG stress have been reported in sorghum (Tounekti *et al.*, 2020) and chickpea (Naim and Qadarif, 2015). In this study, landraces such as ‘M2’ and ‘M1’ maintained higher GRI under moderate stress (-0.2 MPa), suggesting genetic differences in how efficiently seeds adjust their osmotic potential under stress.

4.3. Germination stress tolerance index

The germination stress tolerance index also declined with increasing PEG concentrations, highlighting the sensitivity of cannabis to water deficits during early development (Cole and Zurbo, 2008; Williams and Mundell, 2018; Adesina *et al.*, 2020). This result aligns with findings in wheat where GSTI decreased under drought stress (Mirza *et al.*, 2015). Nonetheless, GSTI varied across landraces: ‘H1’, ‘M2’, and ‘L2’ showed higher tolerance under laboratory conditions, while ‘H1’, ‘B3’, ‘B1’, ‘B2’, and ‘M2’ exhibited improved performance under greenhouse conditions. In some cases, GSTI increased under higher PEG concentrations, suggesting that landrace-specific and environmental factors influenced responses (Razzaq *et al.*, 2017). For example, in ‘H1’ and ‘M2’ the response appeared genetically linked, whereas in other landraces under greenhouse conditions the effects were more environmentally driven. Consistently, ‘L1’ and ‘M1’ exhibited the lowest GSTI, while ‘M2’ showed the highest under laboratory conditions, and ‘B3’ maintained relatively higher values under greenhouse conditions. These results support Giancarla *et al.* (2012), who noted that drought tolerance varies not only between species but also within species. While all germination traits were positively correlated, GSTI consistently showed weaker associations with GP, GRI, and SVI. This weaker relationship may be attributed to variation in seed quality rather than physiological drought resilience. As a result, GSTI was not always a stable measure of drought tolerance in cannabis, unlike the other parameters that were more strongly interrelated. This contradicts

Ahmad *et al.* (2015), who proposed GSTI as a robust criterion for screening stress tolerance. The findings of the current study shows that GSTI may not always serve as a dependable screening tool in cannabis.

4.4. Seedling length

Seedling length followed similar trends to germination traits, decreasing with increasing PEG concentrations. Reduced seedling growth was attributed to lower water potential, which dehydrates protoplasm, reduces turgidity, and limits cell elongation and division (Vijay *et al.*, 2018; Bukhari *et al.*, 2021). Hormonal imbalances, particularly involving cytokinin and abscisic acid, may have further restricted growth. Among the plant hormones, abscisic acid (ABA) and auxin have been found to be critical in the response of hemp to drought stress (Gao *et al.*, 2018). Abscisic acid particularly plays a central role as a stress hormone, accumulating rapidly under drought stress to regulate survival mechanisms (Gao *et al.*, 2018). The ability of certain landraces to sustain longer seedlings under stress, such as ‘M2’ and ‘M1’, may be genetically associated with mechanisms like increased root elongation or hormone regulation, enhancing water uptake.

4.5. Seed vigour index

The seed vigour index, which integrates both germination and seedling growth, was also reduced by PEG treatments. The reductions in SVI were likely due to osmotic effects of PEG disrupting mitotic activity and modifying cell wall properties required for elongation (Vijay *et al.*, 2018). Consequently, exposure to low water potential during early growth stages compromises emergence, seedling establishment, and vigour (Bukhari *et al.*, 2021). Variation in SVI among landraces mirrored trends in germination and seedling length, confirming its sensitivity to drought stress (Bouchyoua *et al.*, 2024). The variation in SVI among landraces

indicates that genetic background contributes to differences in maintaining vigour under drought stress

Overall, the response of cannabis landraces to PEG-induced drought stress varied across conditions and genotypes. Under laboratory conditions, 'L2' recorded the highest germination percentage and rate, while 'B1' exhibited the highest GSTI. In contrast, 'L1' and 'M1' consistently performed poorly. The clustering of 'B1' and 'L2' together in the hierarchical analysis corroborates their shared resilience to laboratory drought stress and suggests possible genetic similarities in drought tolerance mechanisms that distinguish them from susceptible genotypes like 'L1' and 'M1'. In greenhouse experiments, 'H1' showed the highest germination, while 'B3' displayed superior performance across parameters, including GRI, GSTI, seedling length, and SVI. The outlier status of 'B3' in hierarchical clustering indicates distinct genetic traits for drought tolerance, warranting further investigation and use in breeding programs.

The differences in response of landraces under laboratory and greenhouse conditions were partly attributed to the contrasting growth media: compost under greenhouse conditions provided better aeration and water-holding capacity compared to the cotton pads used in Petri dishes under laboratory conditions (Tiawoun *et al.*, 2017). Environmental and genetic makeup differences further amplified these trends, resulting in lower germination under laboratory than greenhouse conditions. Collectively, these findings indicate that while cannabis is generally sensitive to water deficits during germination and early growth, significant variability exists among landraces, with certain genotypes showing partial tolerance under mild stress. This variability provides a crucial foundation for selecting resilient genotypes for breeding programs aimed at enhancing drought tolerance in cannabis.

5. Conclusion

The current study investigated the effect of PEG-induced drought stress on the germination of different cannabis landraces. PEG treatments reduced germination characteristics, including germination percentage, germination rate, germination stress tolerance index, seedling length, and seed vigour index. However, the findings varied with the PEG concentration, landrace, and environment. Landraces ‘L2’, ‘B1’ and ‘B3’ showed drought tolerance amongst the landraces. However, their tolerance depended on the growing environment, with ‘L2’ and ‘B1’ indicating drought tolerance under controlled growing conditions while ‘B3’ exhibited drought tolerance under greenhouse conditions. Thus, these findings indicate that ‘L2’, ‘B1’, ‘H1’, and ‘B3’ may be a suitable choice for farmers facing limited access to climate adaptation resources like irrigation technologies. Furthermore, these landraces may be suitable for further study for breeding programs targeted at the development of drought-resistant varieties. In light of the present findings, future research should explore the impact of drought stress on cannabis landraces in different growth stages, taking into consideration the morphological, biochemical, and genetic traits.

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