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Optimizing Germination and Seedling Vigor in *Verbascum thapsus* L.: Synergistic Effects of Temperature, Media Composition, and Sowing Depth in Controlled Germination

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Abstract

This study, conducted at the Plant Tissue Cultures Laboratory of Kütahya Dumlupinar University's Biology Department, optimised germination protocols for the medicinal plant *Verbascum thapsus* L. using a completely randomised block design with four replicates. ANOVA and Tukey's HSD test ($\alpha = 0.05$) were used for data analysis. Three experimental approaches were employed: standard Petri dish germination, tissue culture-based germination (MS medium), and stratified substrate sowing. Seeds were exposed to varying temperatures (16/22 °C, 25°C), media compositions (pH 5.0–6.5; agar 0.6–0.8%; GA₃ 0–50 ppm), and sowing depths (0.1–2.0 cm) to assess germination parameters and seedling quality. Results indicated that 25°C was superior to 16/22 °C, enhancing both germination rates (58.33%) and seedling vigor index (SVI: 443.38). Optimal MS medium composition was pH 6.0 with 0.6% agar and 25 ppm GA₃ (93.33% germination), with higher GA₃ inhibiting growth. Acidic pH (5.0) promoted root elongation (9.77 cm) but reduced germination. Stratified sowing showed drastically reduced germination (78.89% to 7.78%) and SVI (618.50 to 6.64%) with increasing depth; surface-sown seeds (0.1 cm) exhibited the shortest mean germination time (4.65 days) and best seedling development. Optimal conditions were found to be 25°C with pH-adjusted (5.0–6.0), low-agar (0.6–0.7%) MS medium, combined with shallow sowing. Deep sowing (>0.3 cm) significantly impaired germination and seedling emergence. The study concludes that *V. thapsus* propagation is maximised under 20–25°C, shallow sowing (≤ 0.3 cm), and MS medium with pH 6.0–6.5, 0.6–0.7% agar, and 10–25 ppm GA₃. These findings provide valuable insights for improved cultivation and suggest future research should validate these protocols in field conditions and investigate soil-microbial interactions.

Keywords: *Verbascum thapsus*, Germination Temperature, Tissue Culture, Sowing Depth, Seed vigor.

INTRODUCTION

The genus *Verbascum* (mullein), belonging to the family *Scrophulariaceae*, comprises approximately 366 taxa globally, with Turkey harboring 256 species, 201 of which are endemic. *Verbascum* is distributed

across Central Anatolia, Turkey's Western and Southern regions. The Central Western Anatolia section of the Aegean region, including Kütahya, contains 23 *Verbascum* taxa (Çeçen, 2024; Kolata & Küçük, 2024). *Verbascum thapsus* (common mullein) is a resilient and sustainable crop with significant potential in traditional and modern herbal medicine due to its minimal maintenance requirements and ability to thrive in poor soils (Gupta *et al.*, 2022). Its cultivation offers ecological benefits, such as improving soil health and reducing erosion, making it particularly valuable in regions like Turkish agriculture (Miller *et al.*, 2019; Dieskau *et al.*, 2020). However, maximising its yield requires optimized cultivation methods, as demonstrated in the Trans-Himalayan Ladakh region, where controlled greenhouse cultivation significantly enhanced fresh plant weight (1.95 kg/plant) compared to trench (420 g/plant) or open-air conditions (370 g/plant) (Dolka *et al.*, 2021). To further promote its adoption, educational initiatives, such as cultivation workshops, can empower farmers and entrepreneurs, bridging traditional knowledge with modern agricultural practices (Gupta *et al.*, 2022; Hazman *et al.*, 2024).

A key factor in successful *V. thapsus* cultivation lies in understanding its complex germination requirements. The species exhibits multifaceted germination cues, responding to a combination of environmental factors rather than relying on a single trigger, which contributes to its wide distribution and resilience. Temperature plays a critical role, as germination is inhibited below 10°C and at constant temperatures above 40°C (Semenza *et al.*, 1978). Optimal germination occurs between 20°C and 25°C, though some studies report a broader range of 15°C–25°C (Marquardt *et al.*, 2000). Chilling can overcome winter dormancy, allowing seeds brought to the surface in autumn to germinate early the following spring (Baskin and Baskin, 1981). Humidity also influences germination, with high moisture accelerating the process and low humidity leading to desiccation (Hassan *et al.*, 2012).

Light is another crucial factor, as *V. thapsus* seeds require exposure to light for optimal germination. Seeds buried too deeply (>0.5 cm) exhibit poor germination due to darkness inhibition (Gross, 1984; Marquardt *et al.*, 2000). Natural sunlight significantly enhances germination, with studies reporting 93% germination in light compared to only 35% in darkness (Semenza *et al.*, 1978). Additionally, soil conditions, including pH and fertility, play a vital role. The species thrives in slightly acidic to neutral soils (pH 6.0–7.5), as extreme acidity can inhibit germination by affecting nutrient availability (Cabi *et al.*, 2022). Well-drained, nutrient-rich soils—particularly those with high nitrogen and phosphorus support robust seedling development (Adams *et al.*, 2013).

Beyond natural conditions, seed treatments and growth media can further enhance germination. The Murashige and Skoog (MS) medium, widely used in plant tissue culture, improves germination when adjusted to a pH of 5.8–6.5, optimizing nutrient availability (Hassan *et al.*, 2012; Li *et al.*, 2015). Additionally, gibberellins (GAs), plant hormones essential for seed germination, can break dormancy and promote rapid growth. Concentrations of 50–100 mg/L have been shown to enhance germination rates and seedling vigor by stimulating enzyme production for embryo nourishment (Hassan *et al.*, 2012; Marquardt *et al.*, 2000; Miller *et al.*, 2019).

This study investigates the environmental factors and pretreatments affecting *V. thapsus* germination and dormancy, aiming to improve establishment and growth for both ornamental and medicinal applications. By examining germination responses under varying temperatures, media types, and GA₃ seed pretreatments, the research provides practical insights for enhancing germination rates and seedling vitality. The findings will be valuable for horticulturists, botanists, and agricultural practitioners seeking to optimize *V. thapsus* cultivation in diverse environments, thereby supporting sustainable and resilient agricultural practices.

MATERIALS AND METHODS

1. Study place and Seed Collection

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This thesis study, conducted at the Plant Tissue Cultures Laboratory of Kütahya Dumlupınar University's Biology Department, investigated *V. thapsus* L., a plant of medicinal importance. Seeds were obtained from the Hekim Sinan Medicinal and Aromatic Plants Research Center in 2023 and stored in a cool environment until use.

2. Experimental Setup and treatments

To comprehensively assess the influence of germination medium and temperature on various germination parameters and subsequent seedling quality of *V. thapsus*, a series of three distinct experiments was meticulously performed.

2.1. Standard petri dish germination

The initial experiment aimed to simulate in vitro germination conditions using sterile Petri dishes as the germination environment. To evaluate germination and early seedling growth at 16/22 °C and 25°C, conduct the test following ISTA (2023) guidelines with modifications: Prepare sterile Petri dishes (9 cm diameter) with double-layered blotting paper moistened with distilled water. Place 25 seeds per dish (in quadruplicate per temperature), spacing them evenly to prevent overlap. Seal dishes with parafilm to maintain humidity and incubate under continuous light (to satisfy photoblastic requirements) at two constant temperatures: 16/22 °C ($\pm 1^\circ\text{C}$) and 25°C ($\pm 1^\circ\text{C}$). Monitor germination daily for 30 days, recording radicle emergence (≥ 2 mm) as germination.

2.2. Tissue Culture-Based Germination (MS Medium)

The experiment used a bifactorial approach to assess the germination of *V. thapsus* under controlled conditions. Two temperature regimes (16/22 °C and 25°C) were tested within a tissue culture-based germination framework using MS Medium. This experiment specifically examined how modifications to the MS medium, such as variations in pH (ranging from 5.0 to 6.5), agar concentrations (0.6% to 0.8%), and gibberellic acid (GA₃) supplementation (10, 25 and 50 ppm) affected germination over a 30-day observation period.

2.2.1. Tissue Culture Protocol

Murashige and Skoog (MS) medium was prepared by dissolving 4.30 g/L MS Basal salt, 0.103 g/L MS vitamins, and 30 g/L sucrose in ultrapure water. The pH was adjusted to 5.6-5.7 using 1 M NaOH or HCl, and 7 g/L agar was added as a solidifying agent. The medium was sterilized by autoclaving at 121°C under 1.1 atm pressure for 30 minutes. For GA₃ treatments, filter-sterilized solutions were aseptically added to the cooled medium to achieve final concentrations of 10, 25, and 50 ppm.

2.2.2. Sterilization Procedures

All equipment, including culture vessels and tools, were sterilized using a Hirayama HG-80 autoclave at 121°C for 30 minutes. Seed surface sterilization was performed using 30% sodium hypochlorite solution with a few drops of Tween-20 for 20 minutes, followed by three rinses with sterile distilled water. The laminar flow cabinet (Metisafe MSC-IIA-120) was sterilized with 70% ethanol and UV exposure for 30 minutes before use.

2.2.3. Culture Conditions and Data Collection

Seeds were cultured in 9 cm Petri dishes containing the prepared media variants and incubated at either 16/22 °C or 25°C under a 14-hour photoperiod provided by cool-white LED lights (50 $\mu\text{mol}/\text{m}^2/\text{s}$). Germination was monitored every three days, and radicle emergence ≥ 2 mm was considered successful germination. After 30 days, seedling vigor was assessed by measuring root and shoot lengths using digital calipers and counting fully expanded leaves. Abnormal seedlings were identified and recorded according to ISTA guidelines.

2.2.4. Nomenclature

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This methodology is referred to as the "Bifactorial In Vitro Germination Assay" to reflect the two tested temperatures and multiple media variables examined in the study. The protocol was designed to provide reproducible results while allowing for systematic evaluation of key factors influencing *V. thapsus* germination and early seedling development.

2.2.5. Quality Control Measures

Environmental conditions were monitored daily using calibrated data loggers to ensure temperature and light consistency. Media pH was verified before sterilization, and random samples were checked for contamination throughout the experiment. All measurements were conducted by trained personnel using standardized protocols to minimise observational bias.

This comprehensive methodology enabled rigorous testing of multiple variables while maintaining the precision required for germination studies in controlled environments. The 30-day experimental duration allowed for complete assessment of germination kinetics and early seedling growth characteristics under the various treatment conditions.

2.3. Stratified Substrate Germination Method

This method focuses on the layered sowing technique and growth medium to determine the effect of sowing depth on *V. thapsus* germination. A controlled experiment was conducted using multi-cell seed trays filled with a sterile, well-draining substrate optimized for seed germination and early seedling development. The substrate comprised 70% peat moss, 20% perlite, and 10% vermiculite to provide adequate drainage, aeration, and moisture.

Seeds were sown at six depths: 0.1 cm, 0.3 cm, 0.5 cm, 0.7 cm, 1.0 cm, and 2.0 cm. Each depth was replicated four times, with 25 seeds per replicate (100 seeds per depth). Seeds sown at 0.1 cm were gently pressed onto the surface. For deeper sowing, seeds were carefully covered with precise substrate layers to achieve target depths. Seed trays were maintained under controlled conditions: 20-22°C daytime and 15-18°C nighttime temperatures, and 14-16 hours of light daily. Bottom watering maintained consistent moisture levels without displacing seeds. A clear humidity dome maintained high humidity while allowing adequate light penetration. Germination was monitored every three days for 30 days. Seedlings were classified as normal, abnormal, or non-germinated according to ISTA 2023 guidelines.

3. Seed germination parameters

- Germination percentage (G%) was measured at the end of the test period according to International Seed Testing Association;

$$G \% = \frac{\text{Total number of germinated seeds}}{\text{Total number of evaluated seeds}} \times 100$$

- Mean germination time (MGT) was calculated by using the following equation, where n is the number of seeds which were germinated on day time, D is the number of days counted from the beginning of the germination test.

$$MGT = \frac{\sum(D \times n)}{\sum n}$$

- Seedling vigor parameters: To assess seedling vigor, root length and shoot length were precisely measured and recorded in centimeters (cm). Root length was measured from the root base to the furthest tip, while shoot length was determined from the root-shoot junction to the shoot apex. Additionally, the number of fully expanded cotyledons and true leaves was counted and recorded for each seedling. These measurements were taken at the termination of each experiment (day 30) for all germinated seeds across both temperature treatments (16°C and 25°C). The seedling vigor index (SVI-I) was calculated by using the following formula;

$$SVI-I = \text{root and shoot length (cm)} \times \text{Germination percentage}$$

4. Statistical Analysis

The experimental design followed a completely randomised block design with four replicates per treatment, each containing 25 seeds. Data were analysed using JMP 6 SAS statistical analysis program. F-test and t-test were used to reveal the differences between treatments at $p < 0.05$ level. TUKEY-HSD multiple comparisons were applied to the treatments that were significant in the F-test (JMP, 2023).

RESULTS

The results presented in Table 1 demonstrate that germination temperature significantly influence the germination and early growth traits of *V. thapsus*. Seeds germinated at 25°C exhibited higher germination rates (58.33%), faster mean germination time (1.71 days), and superior seedling vigor compared to those at 16/22 °C, indicating that warmer conditions optimise metabolic processes critical for germination. Figure 1 illustrates the effects of seed germination media treatments on the germination percentage of *V. thapsus* seeds. Therefore, a higher germination temperature of 25°C results increased germination percentages, indicating that warmer conditions favor seed viability and growth. Among the tested media, MS medium pH 6.0 and 0.6% agar produced the most favourable outcomes. Notably, gibberellic acid (GA₃) at 25 ppm substantially improved germination (93.33%), though higher concentrations (50 ppm) had inhibitory effects, suggesting a dose-dependent response (Figure 1). The mean germination time of *V. thapsus* seeds varied between 1.19 ± 0.07 to 2.33 ± 0.08 days. Therefore, the highest mean germination time was recorded in the 6% agar treatment (1.19 ± 0.07 days) (Figure 1).

Table 1: Means of germination percentage (G%), mean germination time (MGT, day), root length (cm), shoot length (cm), leaves number, and seedling vigor index (SVI) as affected by germination temperature on *V. thapsus* seeds.

Measured Parameters	25 °C	16/22 °C
% Germination	$58.33 \pm 2.74^*$	38.95 ± 2.56
Mean germination time (day)	$1.71 \pm 0.06^*$	3.80 ± 0.10
Root length (cm)	$5.22 \pm 0.36^*$	3.49 ± 0.21
Shoot length (cm)	$3.49 \pm 0.14^*$	2.43 ± 0.15
Leaf number	$14.18 \pm 0.44^*$	7.68 ± 0.65
Seedling vigor index	$443.39 \pm 34.27^*$	266.65 ± 18.10

t ($p < 0.05$); $\pm$ SE

Among the various media treatments, those incorporating growth regulators like GA₃ showed significantly enhanced performance, particularly in combinations with different agar concentrations and pH levels. The results suggest that optimising both temperature and media treatments can substantially improve the germination and early growth of *V. thapsus*, highlighting the importance of environmental conditions and nutrient availability in seedling establishment.

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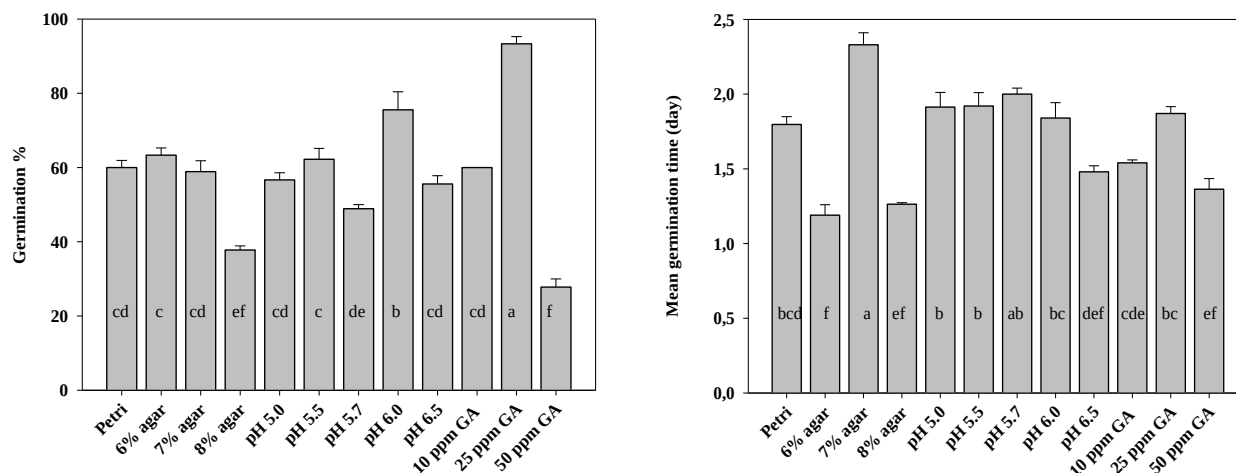


Figure 1: Means of germination percentage and mean germination time as affected by seed germination media treatments on *V. thapsus* seeds. According to the Tukey HSD test, groups with the same letter are not significantly different at $p \leq 0.05$

The interaction effects between germination temperature and media composition on *V. thapsus* seed germination and seedling development reveal critical insights into optimal propagation conditions (Table 2). Root length, shoot length, and leaf number changed significantly with different combinations of mediums. The maximum root length (9.77, 6.63 cm; respectively) was obtained pH 5.0 and 0.6% agar treatments. Except for the petri dish treatment, no statistical significance was observed in shoot length amongst the different treatments. The number of leaves per plant was evaluated, the MS medium with pH 6.0 treatment being the most prolific, with an average of 16.62 ± 0.66 leaves per plant. Notably, GA₃ supplementation at 25 ppm achieved the highest germination rate (93.33%), but this treatment paradoxically reduced root length (3.49 cm) and leaf number (9.33), suggesting a trade-off between germination stimulation and balanced seedling development. Acidic pH (5.0) at 25°C promoted exceptional root elongation (9.77 cm) and SVI (760.5), aligning with *V. thapsus*'s adaptability to slightly acidic conditions.

Table 2: Effect of different treatments on *V. thapsus* seedling parameters (root length, shoot length and number of leaves). According to the Tukey HSD test, groups with the same letter are not significantly different at $p \leq 0.05$

Treatments	Root length (cm)	Shoot length (cm)	Leaf number
Petri (Distilled water)	$1.30 \pm 0.02^{b*}$	0.50 ± 0.39^b	2.17 ± 0.01^d
6% agar	6.63 ± 1.22^{ab}	3.44 ± 0.55^a	15.63 ± 1.05^a
7% agar	5.70 ± 1.51^{ab}	4.00 ± 0.53^a	14.80 ± 0.80^{ab}
8% agar	4.57 ± 1.09^b	3.21 ± 0.27^a	14.71 ± 0.92^a
pH 5.0	9.77 ± 1.03^a	3.73 ± 0.46^a	14.00 ± 0.76^{ab}
pH 5.5	4.17 ± 0.53^b	4.00 ± 0.32^a	14.89 ± 0.65^a
pH 5.7	4.64 ± 0.64^b	3.21 ± 0.33^a	15.43 ± 1.25^a
pH 6.0	4.58 ± 0.67^b	3.73 ± 0.35^a	16.62 ± 0.66^a
pH 6.5	3.75 ± 0.79^b	3.50 ± 0.09^a	15.50 ± 0.45^a
10 ppm GA	6.75 ± 0.06^{ab}	4.38 ± 0.04^a	15.33 ± 0.33^{ab}
25 ppm GA	3.49 ± 0.31^b	3.92 ± 0.06^a	9.33 ± 0.88^{bc}
50 ppm GA	1.78 ± 0.19^b	2.20 ± 0.39^{ab}	4.33 ± 0.33^{cd}

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Conversely, higher agar concentrations (0.8%) consistently underperformed, likely due to restricted water or nutrient mobility. These findings collectively demonstrate that synergistic temperature-media interactions, particularly 25°C with ph-adjusted (5.0–6.0), low-agar (0.6–0.7%) MS media, maximise germination and seedling vigor, while GA₃ should be cautiously dosed (10–25 ppm) to avoid growth imbalances (Table 2).

The data reveal a clear inverse relationship between sowing depth and germination success in *V. thapsus* (Figure 2). Seeds sown at the shallowest depth (0.1 cm) achieved the highest germination percentage (78.89%) and seedling vigor index (SVI, 618.50), along with the shortest mean germination time (4.65 days). In contrast, increasing sowing depth progressively reduced germination rates (58.89% at 0.3 cm to 7.78% at 2.0 cm) and delayed germination (MGT of 9.22 days at 2.0 cm) while suppressing root/shoot growth and leaf development. Notably, even minor depth increases (0.3 cm vs. 0.1 cm) caused significant declines in SVI (374.41 vs. 618.50), underscoring the species' sensitivity to light availability and oxygen diffusion near the soil surface. The near-linear reduction in all measured traits with depth aligns with *V. thapsus*'s ecological adaptation as a pioneer species requiring minimal soil coverage. These findings emphasise that optimal field sowing should prioritise surface-level (≤ 0.3 cm) placement to maximise establishment success, with deeper sowings (>0.5 cm) drastically compromising productivity (Figure 2).

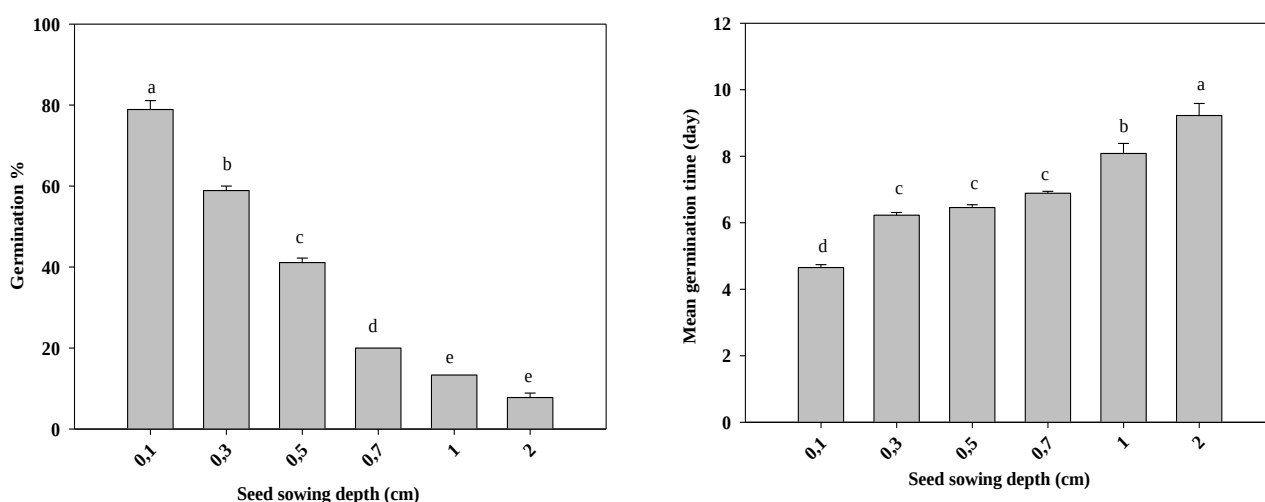


Figure 2: Means of germination percentage and mean germination time as affected by seed sowing depth on *V. thapsus* seeds. According to the Tukey HSD test, groups with the same letter are not significantly different at $p \leq 0.05$

In the present study, a statistically significant relationship was determined at the 0.01 level between seedling root length, stem length, number of leaves, fresh and dry weight in *V. thapsus* at different seed sowing depths. The data presented in Table 3 corroborate and visually reinforce the trends observed in Figure 2, demonstrating a pronounced decline in seedling parameters with increasing sowing depth. In the current study, the length of both the root and shoot of the seedlings was found to decrease in accordance with an increase in seed sowing depth. Surface-sown seeds (0.1 cm) exhibited the highest root length (2.89 cm) and shoot length (4.80 cm). The findings indicated that the optimal conditions for maximising leaf number, fresh and dry weight were obtained at a depth of 0.1 cm. On the contrary, the lowest seedling parameters were determined at 2.0 cm (Table 3).

Table 3: Effect of different seed sowing depth on *V. thapsus* seedling parameters (root length, shoot length, number of leaves, fresh and dry weight). According to the Tukey HSD test, groups with the same letter are not significantly different at $p \leq 0.05$

Seed sowing depth (cm)	Root length (cm)	Shoot length (cm)	Leaf number (adet)	Fresh weight (gr)	Dry weight (gr)
0.1	$2.89 \pm 0.01^{a*}$	4.80 ± 0.12^a	6.25 ± 0.06^a	82.00 ± 0.58^a	8.18 ± 0.05^a
0.3	2.85 ± 0.02^a	3.44 ± 0.01^b	6.06 ± 0.05^a	79.00 ± 0.58^b	7.65 ± 0.10^b
0.5	2.64 ± 0.03^b	3.30 ± 0.06^b	5.59 ± 0.07^b	72.00 ± 0.06^c	7.55 ± 0.04^b
0.7	2.50 ± 0.06^{bc}	3.07 ± 0.03^{bc}	4.35 ± 0.15^c	62.07 ± 0.41^d	6.19 ± 0.11^c
1.0	2.45 ± 0.01^c	2.80 ± 0.12^c	4.18 ± 0.09^c	53.25 ± 1.02^e	5.27 ± 0.05^d
2.0	2.40 ± 0.06^c	2.70 ± 0.12^c	4.00 ± 0.00^c	42.60 ± 0.12^f	4.19 ± 0.08^e

This data reflects optimal light exposure and minimal physical barriers to seedling emergence. In contrast, seeds sown at deeper layers (≥ 0.5 cm) showed a steep reduction in performance, with seedling parameter rates dropping by $\sim 50\%$ at 2.0 cm compared to surface sowing (Table 3). This pattern aligns with *V. thapsus*'s ecological strategy as a ruderal species adapted to colonise disturbed, open habitats where seeds typically remain near the soil surface. Reduced light penetration and restricted gas exchange at greater depths likely impair metabolic activation and resource mobilisation, leading to weaker root and shoot growth. These results highlight the critical importance of precision sowing in cultivation practices, as even minor deviations from optimal depth (0.1–0.3 cm) can significantly compromise establishment success. For field applications, mechanical sowing equipment should be calibrated to ensure shallow placement, while mulching or soil crusting must be mitigated to prevent accidental seed burial.

DISCUSSIONS

Germination and seedling establishment of *V. thapsus*, commonly known as great mullein, are significantly governed by temperature regimes, playing a crucial role in its successful propagation and spread. Research indicates that maintaining constant temperatures is more conducive to germination compared to fluctuating temperatures. Specifically, a stable temperature of 25°C has been observed to promote significantly higher germination percentages, often exceeding 80%, indicating a strong preference for this thermal condition during the initial stages of development. Furthermore, this constant temperature leads to a reduction in the time required for germination, with seedlings emerging within a shorter timeframe of 5 to 7 days. Conversely, fluctuating temperature regimes, such as alternating between 22°C and 15°C , have been shown to result in lower germination rates and extended germination periods (Vanlerberghe & Van Assche, 1986; Seipel *et al.*, 2015).

While the optimal temperature range for *V. thapsus* germination has been identified as lying between 15°C and 25°C (Baskin & Baskin, 2014), temperatures outside this range can have detrimental effects on the germination process. Temperatures below 10°C tend to slow down the metabolic processes essential for germination, leading to a significant delay in emergence, often extending beyond 14 days. This retardation is attributed to the reduced enzymatic activity and overall metabolic inefficiency at lower temperatures. On the other hand, temperatures exceeding 30°C can inhibit germination altogether, resulting in significantly reduced germination rates, typically below 50% (Semenza *et al.*, 1978; Li *et al.*, 2023). This inhibition is likely due to heat stress and the denaturation of critical proteins involved in germination. The presence of light/dark cycles and seasonal temperature variations further influences germination success, demonstrating the plant's ecological adaptability to different environmental conditions (Catara *et al.*, 2016). In addition to affecting germination rates, suboptimal temperatures can also diminish seedling vigor, underscoring the importance of thermal stability for robust early growth and development.

In vitro studies provide further insights into the factors influencing *V. thapsus* germination. Research has demonstrated that Murashige and Skoog (MS) medium, when supplemented with a concentration of 25 mg L⁻¹ of gibberellic acid (GA₃) and adjusted to a pH of 6.0, maximizes germination rates. This formulation provides the necessary nutrients and hormonal stimulation for optimal germination under controlled conditions (Rizk *et al.*, 2023). However, increasing the GA₃ concentration to 50 mg L⁻¹ has been shown to suppress germination, indicating a delicate balance in hormone levels required for successful seed development. It is worth noting that conflicting evidence exists, suggesting that higher GA₃ concentrations, ranging from 50 to 100 mg L⁻¹, may enhance germination in other plant species (Miller *et al.*, 2019; El Ismaili *et al.*, 2022), highlighting the species-specific nature of hormonal responses.

Furthermore, the concentration of agar and the pH of the growth medium are also critical factors in *V. thapsus* germination. An agar concentration ranging from 0.6% to 0.7% is considered optimal, providing sufficient support and moisture without restricting water availability. Maintaining the pH within the range of 5.5 to 7.0 is also crucial, with a pH of 6.5 yielding the shortest germination time of approximately 5.0 days and the highest germination percentage, reaching 100% (Akin, 2021; Sisay & Fentaye, 2023). Studies have shown that root elongation is maximised at a pH of 5.0 with a 0.6% agar concentration, while stem growth and leaf number peak at a pH of 6.0 (Sanavy & Jami Moeini, 2003). Excessive agar concentrations, exceeding 0.7%, can reduce water potential, inducing stress on the developing seedlings and hindering germination (Bayanati *et al.*, 2015). This highlights the importance of carefully balancing the components of the growth medium to provide optimal conditions for germination and early seedling development.

The depth at which seeds are sown also has a profound effect on *V. thapsus* germination and subsequent seedling vigor. Surface sowing, at a shallow depth of approximately 0.1 cm, optimises germination rates, achieving around 85%, and promotes superior seedling traits, including seedling parameters. Conversely, sowing seeds at depths greater than 0.5 cm significantly reduces germination rates to below 30% and delays emergence (Kumar *et al.*, 2024; Ansari & Daehler, 2000). This reduction in germination is likely due to light limitation and increased mechanical resistance encountered at deeper soil layers, such as 2.0 cm, which impair growth (Semenza *et al.*, 1978).

Soil texture also plays a role in modulating these effects. Sandy soils, with their better drainage and aeration, can tolerate sowing depths of up to 0.3 cm, while clay soils, which tend to compact and crust, require shallower sowing depths of approximately 0.1 cm to prevent crusting and facilitate seedling emergence (Baskin & Baskin, 2011). The temperature fluctuations that occur at the soil surface further synchronise germination (Catara *et al.*, 2016; Milberg *et al.*, 2000), emphasising the species' adaptation to surface microhabitats.

Conclusion

In conclusion, for effective *V. thapsus* propagation, it is recommended to maintain constant temperatures within the range of 20–25°C, utilise MS medium with a pH between 6.0 and 6.5, and an agar concentration of 0.6–0.7%, and practice surface sowing at a depth of ≤0.3 cm. These conditions are crucial for balancing metabolic efficiency, ensuring adequate nutrient availability, and maximising light exposure, aligning with the species' inherent ecological niche. Future research efforts should focus on validating these protocols under field conditions and exploring interactions with soil moisture levels and microbial communities to further refine cultivation strategies for medicinal or ecological applications.

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