June 30, 2025

https://ijoabs.com/

DOI: 10.5281/zenodo.

Enhancing Germination and Seedling Vigor Efficiency of *Stachys byzantina* C. Koch: Synergistic Effects of Temperature, Growth Medium, and Seed Treatments

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Received Date: 02-May-2025 **Accepted Date: 12-June-2025** **Published Date: 30-June-2025**

Abstract

This study optimised Stachys byzantina germination protocols using a completely randomised design with four replicates, analysed via ANOVA and Tukey's HSD (α = 0.05). Three germination methods were compared: standard Petri dish, tissue culture (Murashige Skoog medium), and in soil. Seeds were subjected to varying temperatures (16/22 °C, 25 °C) and pretreatments to assess germination parameters and seedling quality. Results indicated that 25 °C significantly improved germination rates (39.83%), root length (4.89 cm), shoot length (4.55 cm), leaves number (6.67), and seedling vigor index (SVI: 396.8) compared to 16/22 °C. Seed treatments generally outperformed untreated seeds. MS medium with GA_3 (10 ppm) and mechanical scarification+100 ppm GA_3 yielded the highest germination percentage and SVI, demonstrating a synergistic effect between hormonal priming and dormancy breaking, promoting superior root and shoot growth. Contrarily, alternating temperatures (16/22 °C) only reduced mean germination time (MGT), while germination rates remained lower than at 25 °C. The germination of S. byzantina in soil at 25 °C significantly enhanced germination efficiency (50.59%) with a notably short MGT (1.80 days), highlighting the temperature's role in balancing germination efficiency and seedling resilience. The findings emphasise the need for temperature-specific protocols, favouring a stable 25 °C, in addition to physical and hormonal treatments for robust establishment versus alternating regimes for rapid germination and seedling vigor in controlled settings.

Keywords: *Stachys byzantina, Germination temperature, Growth medium, Seed treatments, Germination, Seedling vigor.*

INTRODUCTION

Stachys byzantina C. Koch (commonly known as lamb's ear) is a perennial plant native to Turkey, and valued for its ornamental and medicinal uses (Richardson *et al*., 2000; Baskin & Baskin, 2014). Despite its ecological challenges, the species holds significant economic and therapeutic potential (Bahadori *et al*., 2020; Hamedani *et al*., 2023). In traditional medicine, *Stachys* species are used for anti-inflammatory and analgesic purposes, though research on *S. byzantina* remains limited. Its leaves are brewed as aromatic tea in Europe and Asia, attracting interest in the food and beverage industries (Bahadori *et al*., 2020). Pharmacologically, *S. byzantina* exhibits antioxidant, antimicrobial (e.g., against *Staphylococcus aureus*; Kiashi *et al*., 2021), and anti-

hyperpigmentation properties, with applications in cosmetics (Bahadori *et al*., 2020). It also demonstrates neuroprotective effects, including cholinesterase inhibition for Alzheimer's management, α-glucosidase inhibition for antidiabetic activity (Sarikurkcu *et al*., 2016), and lipase inhibition for anti-obesity potential. Extracts show cytotoxic effects against cancer cell lines, suggesting anticancer utility (Jassbi *et al*., 2014), and its phytochemicals aid in managing nematode infestations in agriculture (Nascimento *et al*., 2020). Recent studies highlight its role in addressing oxidative stress, inflammation, diabetes, and infections (Hamedani *et al*., 2023). Thus, *S. byzantina* is a multifaceted species with applications spanning traditional medicine, pharmaceuticals, cosmetics, agriculture, and food industries, underscoring its dual role as both an ecological concern and a resource of modern value.

S. byzantina, a species valued for its ornamental and medicinal uses, primarily propagates through seeds. Optimal growth requires well-drained sandy or loamy soils (pH 6.0–7.5) and moderate temperatures (15–25°C), with germination influenced by environmental and physiological factors such as temperature, moisture, and soil conditions (Baskin & Baskin, 2014). Studies indicate that germination is most successful between alternating temperatures of 15/10°C and 22/15°C, peaking at 55.6% under 22/15°C, while nearly inhibited below 10/5°C (Vojík *et al*., 2022). However, extreme temperatures and poor soil drainage can reduce seed viability or increase disease risks (Finch-Savage & Bassel, 2016). The species exhibits moderate salinity tolerance, though high salt concentrations or prolonged leaching (>96 hours) may hinder germination (Rezvani *et al*., 2021).

Seed dormancy, a critical ecological adaptation, ensures germination occurs under favourable conditions. *S. byzantina* seeds display physiological dormancy, alleviated by cold stratification (e.g., 4°C), which softens seed coats and activates hormonal changes (Vojík *et al*., 2022). Conversely, high temperatures (40–50°C) may induce secondary dormancy (Baskin & Baskin, 2014). The breaking dormancy processes involve various methodologies, including mechanical scarification, gibberellic acid treatments, and alternating temperatures to simulate natural environmental triggers (Bertsouklis *et al*., 2022; Jaganathan & Biddick, 2021). Nutrient availability, particularly during early growth, further enhances establishment, as balanced fertilisers improve yield and resilience in pokeweed and lamb's ear seedlings (Rezvani *et al*., 2021).

Enhancing seed quality, germination rates, and seedling vigor is critical for improving agricultural productivity and food security. Seed priming techniques such as hydropriming, osmopriming, and biopriming pre-treat seeds to activate metabolic processes, significantly boosting germination speed and uniformity, even under stress (Baskin & Baskin, 2014). Genomics has further advanced this field by identifying genes linked to stress tolerance and germination efficiency (Voss-Fels & Snowdon, 2016), while vigor tests provide sensitive assessments of seed performance (Finch-Savage & Bassel, 2016). Cold stratification (4–6 weeks at 4°C) simulate winter conditions, breaking physiological dormancy and synchronizing germination with favorable spring temperatures (Finch-Savage & Bassel, 2016). Wild pear seeds treated with gibberellic acid + stratification for 60 days (93%) reached the maximum germination percentage in a shorter period (Baharvandi *et al*., 2020). Mechanical scarification, involving seed coat abrasion, enhances water uptake in hard-coated seeds (Chichaghare *et al*., 2021).

Optimal soil conditions, such as well-drained, nutrient-rich substrates with organic amendments, hardly improve germination success, alongside regulated temperatures (15–25°C) using germination mats or greenhouses (Baskin & Baskin, 2014). For *S. byzantina*, surface sowing ensures light exposure, a key trigger for germination, while fine misting and light mulching maintain moisture without waterlogging (Vojík *et al*., 2022; Finch-Savage & Bassel, 2016). Smoke exposure and brief heat shocks (≤40°C) further stimulate germination and vigor, though prolonged heat damages seeds (Tavşanoğlu *et al*., 2015). Hormonal treatments, such as low-dose gibberellic acid (GA_3), promote germination, whereas high concentrations inhibit it; mechanical scarification and controlled leaching (≤72 hours) also alleviate dormancy (Rezvani *et al*., 2021).

Pre-soaking seeds in 0.1–0.5% KNO_3 for 24–48 hours enhance dormancy breaking, and field applications (100–200 kg/ha) improve establishment, especially when combined with GA_3 (Baskin & Baskin, 2014; Rezvani *et al*., 2021). Consistent post-treatment moisture is vital for success (Finch-Savage & Bassel, 2016). Integrating these methods, priming, stratification, scarification, and hormonal or chemical treatments,

creates synergistic effects, maximizing germination rates and seedling resilience in *S. byzantina*. This holistic approach supports sustainable cultivation, balancing ecological adaptability with agricultural demand, and underscores the species' dual role as both a horticultural asset and a model for seed technology innovation.

This study examines the effects of germination temperature, growth medium, and seed treatments on *S. byzantina* germination and seedling vigor. The aim is to improve germination rates and seedling vigor for sustainable cultivation in horticulture and agriculture, given its economic importance in herbal medicine and cosmetics.

MATERIALS AND METHODS

1. Seed Collection and Study Location

This research investigated the medicinal plant *S. byzantina* L. at Kütahya Dumlupınar University's Biology Department Plant Tissue Cultures Laboratory. Seeds were acquired from the Hekim Sinan Medicinal and Aromatic Plants Research Center in 2023 and stored under cool conditions until experimentation.

2. Experimental Design and Dormancy Breaking Treatment

A completely randomised design (CRD) was used, featuring four replicates for each treatment combination of two factors; the first factor consists of germination temperature with two levels: 16/22 °C and 25 °C with 16/8 hours light/dark cycles.

Various physical and chemical treatments were applied to disrupt the seed coat and break the seed dormancy. For each treatment, three replicates of ten seeds were considered.

Treatments: Untreated seed (Control): Included sterilised and untreated seeds as a control.

Soaking in distilled water: Seeds were soaked in distilled water at room temperature for 24 hours.

Murashige Skoog Medium (MS): Murashige-Skoog medium was used for the germination of *S. byzantina* seeds.

MS + GA_3 (10 ppm): Seeds were sown in MS medium in the presence of 10 ppm gibberellic acid.

Salicylic acid (SA): Seeds were soaked in 0.25- and 0.50-mM SA for 48 hours.

Potassium nitrate (KNO_3): Seeds were soaked in 10% and 20% KNO_3 for 24 hours.

Mechanical scarification + Soaking in distilled water: *Mechanical scarification* was applied to *S. byzantina* seeds following ISTA (2023) guidelines to overcome physical dormancy. Seeds were inspected for integrity, and tools (fine-grit sandpaper: 120–180 grit or sterile scalpels) were sterilized with 70% ethanol. Scarification was performed by gently abrading seeds between sandpaper sheets for 1–2 minutes or nicking the coat near the hilum, ensuring minimal embryo damage. Post-treatment, seeds were sieved to remove debris and rinsed with distilled water. After mechanical scarification, the seeds were soaked in distilled water for 48 hours.

Mechanical scarification + GA_3: After the mechanical scarification, seeds were soaked for 24 hours in a gibberellic acid water solution (GA_3, 100 ppm) before sowing.

Cold dry stratification: *S*eeds were subjected to cold dry stratification by placing them in sterile, airtight containers and storing them at 4 °C for 30 days. Post-stratification, seeds were equilibrated to room temperature (20 °C) for 24 hours before germination testing.

Cold wet stratification: Seeds were sown in petri dishes on filter paper moistened with sterile distilled water and covered with parafilm. These were refrigerated at 4°C for 30 days, with weekly monitoring for moisture levels.

Thermal shock: This method aligns with general ISTA (2023) guidelines for physical dormancy-breaking in hard-coated seeds. Seeds were exposed to thermal shock by incubating them in a preheated laboratory oven at 40 °C ± 1°C for 10 minutes to disrupt seed coat impermeability. Post-treatment, seeds were immediately cooled to room temperature (20 °C) for 15 minutes to prevent prolonged heat stress.

3. Germination Assessment

Seeds were evaluated using three methods:

3.1. Standard Petri Dish Germination

Seeds were surface-sterilized with 1% sodium hypochlorite (2 minutes), rinsed thrice with distilled water, and placed on sterile filter paper (three layers) in 9 cm Petri dishes moistened with distilled water.

3.2. Murashige and Skoog (MS) Medium

Seeds were sown on MS medium (4.43 g/L basal salts, 30 g/L sucrose, 0.8% agar, pH 5.7) and solidified post-autoclaving (121°C, 15 minutes). For GA_3 treatment on MS medium, filter-sterilised solutions were aseptically added to the cooled medium to achieve final concentrations of 10 ppm GA_3.

For both methods, four replicates of 25 seeds each were incubated under two temperature regimes (16/22°C ± 1°C and 25°C ± 1°C) with an 8/16-hour light/dark photoperiod (cool-white fluorescent light, 35 $\mu mol \cdot m^{-2} \cdot s^{-1}$). Germination (radicle emergence ≥2 mm) was recorded daily for 30 days. Moisture levels in Petri dishes were maintained with distilled water, while MS plates were sealed to prevent desiccation. Data on germination percentage, mean germination time (MGT), and index were analyzed using ISTA (2023) compliant protocols, with untreated seeds as controls.

3.3. The Process of Germination in Soil

Seeds were evaluated under a fixed temperature of 25°C ± 1°C to assess germination performance. Surface sowing (0.3 cm depth) was selected as the optimal depth based on preliminary trials, where deeper sowing (>0.3 cm) suppressed germination due to light requirements. Seeds were sown in sterile multi-cell trays filled with a soil (70% peat moss, 20% perlite, 10% vermiculite), chosen for its drainage and moisture retention properties. Each cell contained one seed to avoid competition, with four replicates of 25 seeds (100 seeds total). Trays were placed in a growth chamber under 16-hour light/8-hour dark photoperiods (cool-white LEDs, 35 $\mu mol \cdot m^{-2} \cdot s^{-1}$) to simulate natural light conditions. Moisture was maintained via bottom watering to prevent seed disturbance, and humidity domes (removed after 7 days) ensured 70–80% relative humidity. Germination (radicle emergence ≥2 mm) and further seedling assessments were recorded after 30 days.

3.4. Seed Germination Assessments

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

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

- Mean germination time (MGT) was calculated using the equation: $MGT = \Sigma(nxD)/\Sigma n$, where n is the number of seeds germinated on each day, and D is the number of days from the beginning of the test.

- Seedling vigor was assessed by measuring root and shoot length (cm) and counting fully expanded cotyledons and true leaves after each 30-day experiment. Root length was measured from the root base to the tip, and shoot length from the root-shoot junction to the shoot apex. These measurements were taken for all germinated seeds in both temperature treatments (16°C and 25°C), and used to calculate the seedling vigor index;

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

4. Statistical Analysis

A completely randomised block design with four replicates of 25 seeds per treatment was used. Data were analysed using JMP 6 SAS statistical analysis software. F-test and t-test were used to reveal the differences between treatments at a $p<0.05$ level (JMP, 2023).

RESULTS and DISCUSSION

Data presented in Table 1 indicated that the germination of *S. byzantina* seeds was significantly influenced by temperature regimes, with seeds incubated at a stable 25°C exhibiting a higher germination percentage (39.83% ± 1.38) compared to those under alternating 16/22°C (33.42% ± 0.83). However, the mean germination time (MGT) was slightly prolonged at 25 °C (8.01 ± 0.13 days). Post-germination growth metrics, including root length (4.89 ± 0.23 cm), shoot length (4.55 ± 0.22 cm), leaf number (6.67 ± 0.23), and seedling vigor index (SVI-I: 396.8 ± 32.08), were significantly enhanced at 25 °C ($p < 0.05$). These findings align with broader studies indicating optimal germination for *S. byzantina* within 15–25 °C (Baskin & Baskin, 2014; Bewley *et al*., 2013; Vojík *et al*., 2022), though Brändel (2006) notes fluctuating temperatures (10°C amplitude at 22°C mean) can improve germination rates. The marginal delay in MGT at 25°C likely reflects metabolic

prioritization: stable warmth optimizes enzymatic activity for sustained seedling development, whereas alternating temperatures may expedite initial imbibition at the cost of vigor (Finch-Savage & Bassel, 2016). This trade-off underscores temperature's dual role in regulating germination efficiency and seedling resilience, emphasizing the need for protocol specificity stable 25°C for robust establishment versus alternating regimes for rapid germination in controlled settings.

Table 1: Means of Germination Percentage (G%), Mean germination time (MGT day), Root length (cm), Shoot length (cm), Number of leaves, and Seedling vigor index (SVI-I) as affected by germination temperature (16/22 ˚C and 25 ˚C) treatments on the in vitro germination traits of *Stachys byzantina* seeds.

Measured Parameters	25 ˚C	16/22 ˚C
Germination percentage (%)	39.83 ± 1.38*	33.42 ± 0.83
Mean germination time (days)	8.01 ± 0.13	7.46 ± 0.14*
Root length (cm)	4.89 ± 0.23*	3.92 ± 0.22
Shoot length (cm)	4.55 ± 0.22*	3.69 ± 0.22
Number of leaves	6.67 ± 0.23*	5.72 ± 0.24
Seedling vigor index (SVI-I)	396.8 ± 32.08*	263.23 ± 20.51

* t ($p<0,05$); ± SE

The interaction between germination temperature and seed treatments played a pivotal role in modulating *S. byzantina* germination success, with pronounced variability across treatment-temperature combinations (Fig. 1). The highest germination rates were achieved under a stable 25°C regime when combined with MS medium + GA_3 (10 ppm) or mechanical scarification + 100 GA_3, underscoring the synergistic effect of hormonal priming and stable warmth. At 25°C, GA_3 likely optimised enzymatic activity (e.g., α-amylase) and metabolic pathways critical for breaking physiological dormancy, while mechanical scarification enhanced water and oxygen uptake by disrupting physical barriers in the seed coat (Baskin & Baskin, 2014; Rezvani *et al*., 2021). This aligns with studies showing that GA_3 stimulates gibberellin signalling, promoting cell elongation and reserve mobilization under thermally stable conditions (Taiz & Zeiger, 2010).

Conversely, thermal shock (40 °C) and dry cold stratification suppressed germination at 25°C, likely due to compounded stress: thermal shock may have denatured proteins or damaged embryos, while insufficient cold stratification duration failed to fully alleviate physiological dormancy (Bertsouklis *et al*., 2022). However, the overall inferior efficacy of alternating temperatures compared to stable 25°C suggests that metabolic processes in *S. byzantina* prioritize steady warmth for sustained growth over rapid germination. This trade-off mirrors findings in other Lamiaceae species, where stable temperatures improve seedling vigor despite minor delays in germination initiation (Finch-Savage & Bassel, 2016). The success of GA_3-based protocols at 25°C reinforces the importance of aligning hormonal treatments with species-specific thermal optima. For instance, GA_3's role in upregulating hydrolytic enzymes (e.g., proteases, lipases) is temperature-dependent, with 25°C likely providing the ideal kinetic energy for enzymatic reactions (Emamipoor & Maziah, 2014). In contrast, KNO_3's moderate efficacy under alternating temperatures may reflect its role as a nitrification source rather than a dormancy-breaking agent, necessitating complementary hormonal or physical interventions for full dormancy alleviation (Rezvani *et al*., 2021). Bertsouklis *et al*. (2022) and Jaganathan & Biddick (2021) found that scarification and GA_3 effectively mimic natural cues for dormancy release and promote germination.

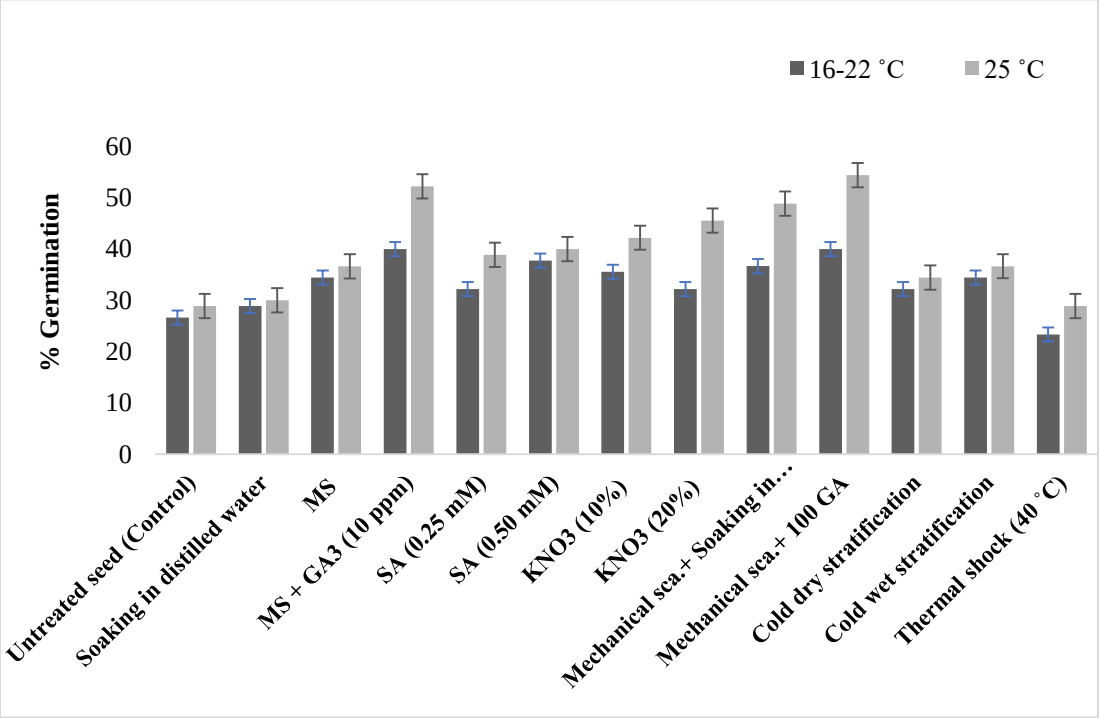


Figure 1: Effect of temperature and different treatments on germination percentage of *S. byzantina* seeds.

Germination temperature and seed treatments significantly interacted to influence the mean germination time (MGT) of *S. byzantina* (Fig. 2). The shortest MGT (~7.0 days) was achieved at alternating 16/22°C with combined treatments of MS medium + GA_3 (10 ppm) and mechanical scarification + GA_3, demonstrating the synergistic effects of hormonal priming and physical dormancy-breaking in accelerating metabolic processes. GA_3 likely enhances enzymatic activity and reserve mobilization under stable thermal conditions, while scarification promotes rapid water uptake by overcoming seed coat impermeability (Baskin & Baskin, 2014; Rezvani et al., 2021). This aligns with the temperature-dependent nature of GA_3-mediated cell elongation and hormonal signalling, which peaks at 25°C (Taiz & Zeiger, 2010). In contrast, untreated seeds at 16-22 and 25 °C (9.3 ± 0.06 and 8.8 ± 0.36 days, respectively) and thermal shock at 25 °C exhibited prolonged MGT. This reflects persistent physiological dormancy in untreated seeds and likely cellular damage or induced secondary dormancy from thermal shock (Bertsouklis *et al*., 2022). This highlights the sensitivity of *S. byzantina* seeds to extreme pre-treatments that may irreversibly compromise viability. Alternating temperatures (16/22°C) only marginally reduced MGT, while germination rates remained lower than at 25°C. This suggests that fluctuating temperatures can partially alleviate dormancy by mimicking natural cues, but lack the stable conditions required for synchronised germination (Rezvani *et al*., 2021). KNO_3's limited efficacy under alternating temperatures likely stems from its role as a nitrification stimulant rather than a primary dormancy-breaking agent, necessitating complementary hormonal treatments for full activation (Berry *et al*., 2021). The temperature-dependent effectiveness of scarification emphasises the need for protocol specificity. While scarification improves water uptake, its full potential is realised only when paired with a stable 25°C temperature, which optimises enzymatic repair of the seed coat and subsequent metabolic activity (Bewley & Black, 1994).

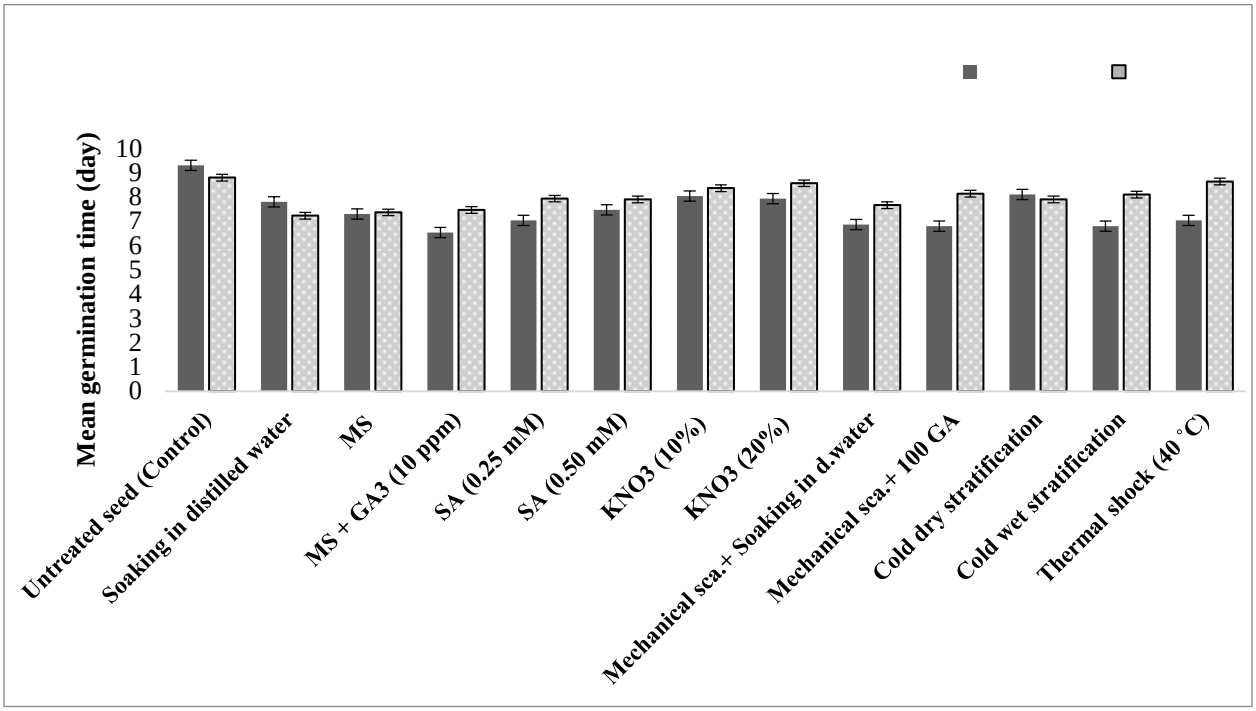


Figure 2: Effect of temperature and different treatments on mean germination time (day) of *S. byzantina* seeds.

In *S. byzantina*, seedling vigor index (SVI-I) was significantly influenced by the interaction of germination temperature and seed treatments (Fig. 3). The highest SVI-I values were achieved with MS medium + GA_3 (10 ppm) at a stable 25°C, suggesting synergistic metabolic activation under optimal warmth. GA_3 likely promoted cell elongation and alleviated dormancy, enhancing the gibberellin-mediated hydrolysis of storage reserves for rapid root and shoot development (Taiz & Zeiger, 2010). Similarly, mechanical scarification + GA_3 amplified vigor at 25°C by improving seed coat permeability and enabling synchronised metabolic activation (Baskin & Baskin, 2014). These findings align with previous research showing that hormonal priming at stable temperatures optimizes seedling establishment over germination speed (Finch-Savage & Bassel, 2016). Conversely, thermal shock (40°C) and dry cold stratification resulted in the lowest SVI-I values at 25°C, likely due to compounded stress: thermal shock potentially denaturing proteins or impairing mitochondrial function, while insufficient cold stratification incompletely breaking dormancy (Bertsouklis *et al*., 2022).

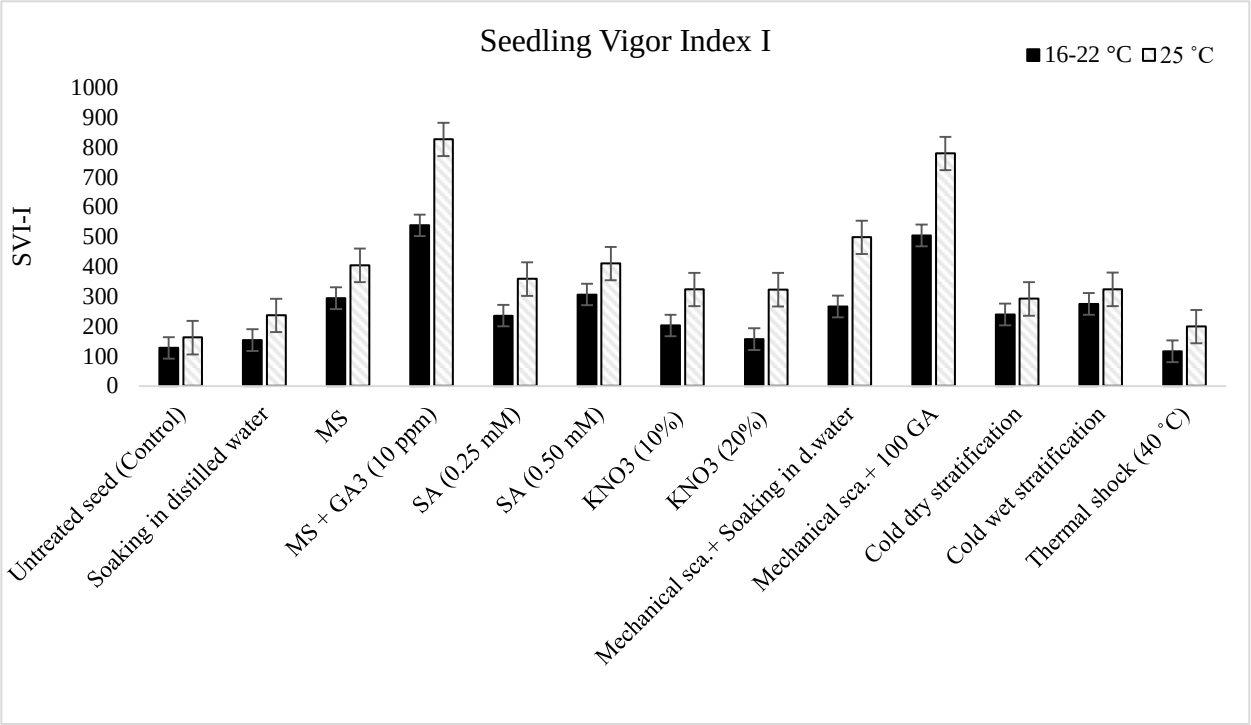


Figure 3: Means of seedling vigor index as affected by the interaction between germination temperature and seed treatments on *S. byzantina* seeds.

The contrast highlights the vulnerability of *S. byzantina* to inappropriate temperature-treatment combinations. KNO_3 (10–20%) showed temperature-dependent efficacy, with modest SVI-I improvements under alternating 16/22°C but inferior performance compared to hormonal treatments at 25°C (Fig. 3). This suggests that KNO_3, acting as a nitrification stimulant (Berry *et al*., 2021), partially alleviates dormancy under fluctuating conditions but lacks the hormonal signalling needed for sustained growth at a stable temperature. The limited vigor observed with KNO_3 underscores the importance of combining nitrogen-based treatments with hormonal or physical interventions for complete dormancy alleviation (Rezvani *et al*., 2021).

The germination of *S. byzantina* in soil was significantly enhanced at 25°C. The results in Table 2 show that the germination percentage was 50.59 ± 4.24%, and the mean germination time was exceptionally short (1.80 ± 0.19 days) (Table 2). This rapid imbibition and metabolic activation can be attributed to the substrate's optimised physical properties; superior aeration, drainage, and moisture retention, which align with findings by Hernández & Hobbie (2010), Yerima *et al*. (2015), Hazarika *et al*. (2023) on the benefits of soil-vermiculite mixes for moisture regulation. The substrate's neutral pH (likely within the ideal 6.0–7.5 range for *S. byzantina*; Baskin & Baskin, 2014) and sterilization likely minimized pathogenic risks, further supporting germination success. However, seedling vigor metrics (root length 6.30 ± 0.71 cm; shoot length 2.97 ± 0.26 cm; leaf number 4.95 ± 0.25) lagged behind hormone-augmented protocols (e.g., MS+GA_3), underscoring the substrate's role in accelerating germination but not fully replicating the growth-promoting effects of hormonal priming. The substrate's performance aligns with studies emphasizing soil quality's critical role in seed vigor (Farooq *et al*., 2011). Its organic-rich composition likely provided initial nutrient access, yet the absence of supplemental hormones or prolonged nutrient availability limited post-germination growth. This mirrors observations by Rezvani *et al*. (2021), where substrate quality alone could not overcome physiological dormancy without targeted treatments. Additionally, the substrate's structure likely facilitated light penetration, critical for *S. byzantina*'s photoblastic germination, as surface-sown seeds received adequate light to trigger metabolic responses.

Table 2: Germination percentage, mean germination time, root length, shoot length and number of leaves of *S. byzantina* seeds germinated at 25 °C in soil

Measured Parameters	25 ˚C
Germination percentage (%)	50.59 ± 4.24
Mean germination time (days)	1.80 ± 0.19
Root length (cm)	6.30 ± 0.71
Shoot length (cm)	2.97 ± 0.26
Number of leaves	4.95 ± 0.25

CONCLUSION

Optimal *Stachys byzantina* germination and seedling vigor depend on carefully integrated temperature, seed treatments, and substrate conditions. Stable 25°C temperature, combined with GA_3 priming (10 ppm) and scarification, yielded the highest germination and seedling vigor, demonstrating the importance of dormancy-breaking. Conversely, thermal shock (40°C) reduced germination, indicating thermal sensitivity. While alternating temperatures (16/22°C) slightly improved germination with KNO_3, stable warmth promoted superior post-germination vigor. Soil accelerated germination but compromised seedling development compared to hormone treatments, highlighting a speed-robustness trade-off. Light exposure and shallow sowing were necessary for germination, whereas excessive leaching or low pH induced secondary dormancy, necessitating precise substrate management.

Increasing the germination of *S. byzantina* is crucial for enhancing its agricultural and nutritional potential and also capitalising on its medicinal and ornamental uses. This perennial herb, recognised for its rich nutritional profile, can significantly contribute to sustainable food systems. Improving germination rates can lead to better crop yields and promote plants use as an unconventional food source. Future studies should investigate field performance under optimised conditions and the molecular mechanisms driving GA_3-mediated metabolic synergy.

REFERENCES

Bahadori, M., Maggi, F., Zengin, G., Asghari, B., & Eskandani, M. (2020). Essential oils of hedgenettles (*Stachys inflata*, *S. lavandulifolia*, and *S. byzantina*) have antioxidant, anti-Alzheimer, antidiabetic, and anti-obesity potential: A comparative study. *Industrial Crops and Products*, *145*, 112089. https://doi.org/10.1016/j.indcrop.2020.112089

Baharvandi, S., Alvaninejad, S., & Balouchi, H. (2020). Effect of cold stratification and gibberellic acid on seed germination of wild pear (*Pyrus glabra* L.) from two different provenances. *Iranian Journal of Forest and Range Protection Research*, *28*, 161–172. https://doi.org/10.22092/ijrfpbgr.2020.341560.1358

Baskin, C. C., & Baskin, J. M. (2014). Seeds: Ecology, biogeography, and evolution of dormancy and germination. Academic Press. https://doi.org/10.1016/B978-0-12-408101-7.00001-8

Berry, N., Shukla, A., & Barkade, E. (2021). Pre-sowing treatment of seeds and its impact on germination of *Gmelina arborea* Roxb. *The Pharma Innovation Journal*, *10*(10), 239–243. https://doi.org/10.22271/tpi.2021.v10.i10d.8004

Bertsouklis, K., Vlachou, G., Trigka, M., & Papafotiou, M. (2022). *In vitro* studies on seed germination of the Mediterranean species *Anthyllis barba-jovis* to facilitate its introduction into the floriculture industry. *Horticulturae, 8*(10), 889. https://doi.org/10.3390/horticulturae8100889

Bewley, J. D., & Black, M. (1994). *Seeds: Physiology of development and germination* (2nd ed.). Plenum Press.

Bewley, J. D., Bradford, K. J., Hilhorst, H. W. M., & Nonogaki, H. (2013). *Seeds: Physiology of development, germination, and dormancy* (3rd ed.). Springer.

Brändel, M. (2006). Effect of temperatures on dormancy and germination in three species in the *Lamiaceae* occurring in northern wetlands. *Wetlands Ecology and Management, 14*, 11–28. https://doi.org/10.1007/s11273-005-1548-5

Chichaghare, A.R., Sreejith, M.M., Reshma, M.R., Abijith, R., Shahina, N.N. and Bhawane, A.K. (2021). Seed Treatment: Breaking dormancy of seeds. Recent trends in propagation of forest and horticultural crops. Taran Publication, New Delhi- 110059.

Emamipoor, Y., & Maziah, M. (2014). An efficient method in breaking of dormancy from *Bunium persicum* (Boiss) Fedtsch seeds: A valuable herb of Middle East and Central Asia. *Asian Pacific Journal of Tropical Biomedicine, 4*(8), 642–649. https://doi.org/10.12980/APJTB.4.2014APJTB-2014-0042

Farooq, M., Hussain, M., & Siddique, K. H. M. (2011). Drought stress in plants: An overview. *Plant Physiology, 1*(1), 1–10. https://doi.org/10.1007/s11845-011-0205-0

Finch-Savage, W. E., & Bassel, G. W. (2016). Seed vigour and crop establishment: Extending performance beyond adaptation. *Journal of Experimental Botany, 67*(3), 567–591. https://doi.org/10.1093/jxb/erv490

Hamedani, M. P., Aram, F., Hadjiakhoondi, A., Samadi, N., Tavakoli, S., Kiashi, F., Goodarzi, S., & Tofighi, Z. (2023). Elucidation of apigenin derivatives from ethyl acetate fraction of *Stachys byzantina* with potent antioxidant and anti-*Staphylococcus aureus* properties. *Traditional and Integrative Medicine*. https://doi.org/10.18502/tim.v8i2.13082

Hazarika, M., Saikia, J., Gogoi, S., Kumar, P., Handique, K., Patel, S. S., & Barooah, L. (2023). Effect of different seedling growing media on tomato and cabbage seedling. *International Journal of Plant & Soil Science, 35*(22), 359–372. https://doi.org/10.9734/ijpss/2023/v35i224144

Hernández, D. L., & Hobbie, S. E. (2010). The effects of substrate composition, quantity, and diversity on microbial activity. *Plant and Soil, 335*, 397–411. https://doi.org/10.1007/s11104-010-0428-9

ISTA (2023). *International rules for seed testing* (2023 ed.). Bassersdorf, Switzerland. https://www.seedtest.org

Jaganathan, G. K., & Biddick, M. (2021). Experimental warming hastens physical dormancy break and germination in tropical Fabaceae. *Frontiers in Plant Science, 12*. https://doi.org/10.3389/fpls.2021.782706

Jassbi, A., Miri, R., Asadollahi, M., Javanmardi, N., & Firuzi, O. (2014). Cytotoxic, antioxidant and antimicrobial effects of nine species of woundwort (*Stachys*) plants. *Pharmaceutical Biology*, *52*(1), 62–67. https://doi.org/10.3109/13880209.2013.810650

JMP (2023). *JMP Pro* (Version 17). SAS Institute Inc.

Kiashi, F., Hadjiakhoondi, A., Tofighi, Z., Khanavi, M., Ajani, Y., Koulaei, S., & Yassa, N. (2021). Compositions of essential oils and some biological properties of *Stachys laxa* Boiss. & Buhse and *S. byzantina* K. Koch. *Research Journal of Pharmacognosy*. https://doi.org/10.22127/RJP.2021.257340.1641

Nascimento, D., Pollo, S., Silva, E., Fernandes, J., Zero, A., Ferreira, R., Braz, L., & Soares, P. (2020). First report of *Meloidogyne incognita* infecting *Stachys byzantina* in Brazil. *Plant Disease*. https://doi.org/10.1094/PDIS-07-20-1628-PDN

Rezvani, M., Amini Bengar, J., & Nikkhahkouchaksaraei, H. (2021). Seed dormancy breaking and germination of pokeweed and lamb's ear: Two invasive plants. *Annali di Botanica, 11*, 67–76. https://doi.org/10.13133/2239-3129/16545

Richardson, D. M., Pyšek, P., Rejmánek, M., Barbour, M. G., Panetta, F. D., & West, C. J. (2000). Naturalization and invasion of alien plants: Concepts and definitions. *Diversity and Distributions, 6*(2), 93–107. https://doi.org/10.1046/j.1472-4642.2000.00083.x

Sarikurkcu, C., Kocak, M., Uren, M., Calapoğlu, M., & Tepe, A. (2016). Potential sources for the management of global health problems and oxidative stress: *Stachys byzantina* and *S. iberica* subsp. *iberica* var. *densipilosa*. *European Journal of Integrative Medicine, 8*, 631–637. https://doi.org/10.1016/j.eujim.2016.04.010

Taiz, L., & Zeiger, E. (2010). *Plant physiology* (5th ed.). Sinauer Associates.

Tavşanoğlu, Ç., Çatav, Ş., & Özüdoğru, B. (2015). Fire-related germination and early seedling growth in 21 herbaceous species in Central Anatolian steppe. *Journal of Arid Environments, 122*, 109–116. https://doi.org/10.1016/j.jaridenv.2015.06.017

Vojík, M., Kadlecová, M., Kutlvašr, J., Pergl, J., & Berchová Bímová, K. (2022). Two shades of grey: Effect of temperature on seed germination of the escaping ornamental species *Lychnis coronaria* and *Stachys byzantina*. *Research Square*. https://doi.org/10.21203/rs.3.rs-1211843/v1

Voss-Fels, K., & Snowdon, R. J. (2016). Understanding and utilizing crop genome diversity via high-resolution genotyping. *Plant Biotechnology Journal, 14*(4), 1086–1094. https://doi.org/10.1111/pbi.12456

Yerima, B., Yerima, B. P. K., Tiamgne, Y., Foko, L., Tziemi, T. C. M. A., & Van Ranst, E. (2015). Effect of substrates on germination and seedling emergence of sunflower (*Helianthus annuus* L.) at the Yongka Western Highlands Research/Garden Park, Bamenda, Cameroon. *TROPICULTURA, 33*(2), 91–100.