

Article ID: 207013
DOI: 10.5586/aa/207013

Publication History

Received: 2024-12-14
Accepted: 2025-06-11
Published: 2025-06-27

Handling Editor

Ewa Muszyńska-Sadłowska;
Warsaw University of Life
Sciences-SGGW, Warsaw,
Poland; [https://orcid.org/
0000-0003-0419-4597](https://orcid.org/0000-0003-0419-4597)

Authors' Contributions

LM: Research concept and design; LM, SZ, ZM, PA, AK, ÉD, GP: Collection and/or assembly of data; JF: Data analysis and interpretation; LM, GP: Writing the article; BC: Critical revision of the article; LM, SZ, ZM, PA, AK, ÉD, BC, JF, GP: Final approval of the article

Funding

This research received no external funding.

Competing Interests







No competing interests have been declared.

Copyright Notice

© The Author(s) 2025. This is an open access article distributed under the terms of the [Creative Commons Attribution License](https://creativecommons.org/licenses/by/4.0/), which permits redistribution, commercial and noncommercial, provided that the article is properly cited.

ORIGINAL RESEARCH

First step from an arable weed to a honey crop: Breaking seed dormancy of *Stachys annua*

László Magyar ¹, Sándor Zsebő ², Zoltán Molnár ²,
Petra Amtmann¹, Attila Kovács ³, Éva Dunai¹, Bálint Czúcz ⁴,
Judit Fehér⁵, Gyula Pinke ^{1*}

¹ Department of Water Management and Natural Ecosystems, Albert Kázmér Faculty of Agricultural and Food Sciences, Széchenyi István University, Hungary

² Department of Plant Sciences, Albert Kázmér Faculty of Agricultural and Food Sciences, Széchenyi István University, Hungary

³ Department of Biosystems and Food Engineering, Albert Kázmér Faculty of Agricultural and Food Sciences, Széchenyi István University, Hungary

⁴ Norwegian Institute for Nature Research, Norway

⁵ KVANN - Norwegian Seed Savers, Norway

*To whom correspondence should be addressed. Email: pinke.gyula@sze.hu

Abstract

Stachys annua (L.) L. – a typical annual weed species in stubble fields – was the most important melliferous plant in the Carpathian Basin during the 19th century. The agricultural intensification led to a drastic decline in the species, and previous efforts for its cultivation were unsuccessful due to its unevenly germinating seeds. This study aims to identify an effective method for overcoming the primary seed dormancy of *S. annua*. In laboratory experiments, we evaluated the effects of moist stratification for 4 weeks (in cold/warm sand) as well as using a gibberellic acid (GA) solution (250 mg/L) on seed germination under two light-temperature regimes (a “constant” regime at 20°C in continuous darkness, and a “fluctuating” regime with 14 h light at 20°C followed by 10 h dark at 10°C). Our results indicate that freshly matured seeds were mostly dormant at maturity. Gibberellic acid has a substantial role in breaking seed dormancy and can help substitute for the cold requirement. The best combination consisted of a GA treatment following a short (4 weeks) warm stratification, which led to a high (98%) germination rate in darkness at 20°C. The results indicate that, under natural circumstances, the seeds of *S. annua* require a longer period for their primary dormancy to be released. Our findings can establish the basis for the development of a dormancy-breaking technology to achieve uniform germination allowing future cultivation of the plant in bee gardens and arable fields.

Keywords

germination; gibberellic acid; seed dormancy; stratification

1. Introduction

For annual crop species, the loss of seed dormancy is among the first signs of domestication. This trait is actively selected against by cultivation itself: lineages that tend to lay dormant will not contribute to the following harvest and thus will not be re-sown the following year (Fuller & Allaby, 2018; Hammer, 1984; Spengler, 2020). The breaking of seed dormancy is still one of the major tools to facilitate breeding programs for releasing new germplasms (Abbasi, 2020; Yalamalle et al., 2024), utilizing or domesticating wild species (Andriamparany & Buerkert, 2019; Khan et al., 2023; Singh et al., 2020; Zhao et al., 2024), to extinguish heterogeneous germination in medicinal plants

(Ekren et al., 2024), and even for the preservation of uncommon species (Cuenca Lombraña et al., 2024; Kırmızı, 2023; Kołodziejek, 2018; Krasnylenko et al., 2023; Santiago et al., 2023).

Stachys annua (L.) L., a species from the Lamiaceae family, is native to the Anatolian-Armenian region, from where it was introduced in Europe as an archaeophyte plant (Csiky et al., 2023), and turned out to be a characteristic weed species of stubble fields (Skrajna et al., 2018; Pinke et al., 2021). In Central Europe, the development cycle of *S. annua* begins in late spring, in the lowest vegetation layer; however, it only flowers due to the larger light input after cereal stands are harvested (Pinke & Pál, 2009). Its most popular Hungarian name, ‘tarlóvirág’, which literally means ‘stubble-flower’, also hints to its most typical flowering site (Berkó & Kardos, 1937; Juhász, 1983; Pinke et al., 2021).

About one and a half centuries ago, *S. annua* was the most important honey plant in the Carpathian Basin. In favourable conditions at the peak of flowering, rich stands of *S. annua* were documented to yield up to 8 kg of honey per bee colony per day, and even seasonal average yields could reach 2–3 kg/day/colony (Berkó & Kardos, 1937; Juhász, 1983; Pinke et al., 2021). The nectar production by *S. annua* can however highly depend on weather conditions (Pinke et al., 2021). As a good quality product, its ample and characteristic monofloral honey used to be a “world brand” of Hungarian apiculture (Pinke et al., 2021). Facing an initial decline in its spontaneous populations, in the early 20th century, there were several well-documented efforts for “saving” this highly esteemed plant by taking it into cultivation (Pinke et al., 2021). Then, in the 20th century, the continued agricultural intensification resulted in a drastic population collapse, turning *S. annua* into a floristic rarity (Pinke, 2020; Pinke et al., 2021). The early attempts for cultivation were greatly hindered by the low germination rates of harvested seeds, which tended to lay dormant in the soil for several years (Pinke et al., 2021). For efficient cultivation, this dormancy must be eliminated through the invention of applicable seed treatments that can break it.

Although similar seed germination behaviours have already been reported for several, mainly biennial or perennial species of the genus *Stachys*, including *S. alpina* (Pinfield et al., 1972, 1975), *S. byzantina* (Rezvani et al., 2021), *S. germanica* (Dunn, 1997; Güleriyüz et al., 2011), *S. mouretii* (Ismaili et al., 2023), *S. palustris* (Brändel, 2006), and *S. sylvatica* (Graae et al., 2009; Slade & Causton, 1979), the seed dormancy release and germination requirements of *S. annua* have not been studied yet.

Pinfield et al. (1972) achieved 90–95% germination proportion of *S. alpina* L. when seeds were treated with 104 ppm GA and kept in constant darkness at 20°C for 12 days. It was observed that higher concentrations of GA increased the germination rate but did not affect the final germination. However, reduced concentrations of GA resulted in lower germination percentage. No germination was observed in distilled water treatments or after stratification for less than 4 weeks. Their studies showed that storage in wet sand for 12–20 weeks was necessary to produce low germination (20–30%). Thus, in *S. alpina*, almost complete germination could be achieved with GA treatment, whereas stratification was only partially effective (Pinfield et al., 1972). This is due to the relatively slow increase in endogenous gibberellic acid levels in freshly ripened, dormant seeds of *S. alpina* during post-ripening (wet chilling), which can be accelerated by the application of external GA (Pinfield et al., 1975). Slade & Causton (1979) investigated the effects of five dormancy-breaking treatments – potassium nitrate, scarification, ethylene, light, and stratification – on *S. sylvatica* L. seeds in a full factorial experiment. It was found that germination occurred in about two-thirds of the different factor combinations, but germination rates were generally low. The best germination (70%) was achieved by stratification at 5°C for 6 weeks, with scarification being inhibited. Thompson & Grime (1979) recorded that seeds of *S. sylvatica* stored dry at 5°C required a large diurnal temperature fluctuation (9°C) to achieve the maximum germination %. Graae et al. (2009) found that fresh seeds of *S. sylvatica*, collected from a range of sites across central and northwestern Europe, had a very low germination percentage (0–19%). After 18 weeks of cold stratification followed by 6 weeks of warm incubation (14 h light/20°C and 10 h dark/10°C), germination ranged from 13% to 54%. When seeds were exposed to 6 weeks of warm incubation

followed by 18 weeks of cold incubation and finally 6 weeks of warm incubation, germination was increased to 50–91%. Brändel (2006) observed that germination of *S. palustris* L. seeds took place between 12–36°C. Optimal germination was recorded at constant temperatures of 24–33°C if the seeds had been stratified (10 weeks at 5°C); however, germination increased under increasing diurnal temperature fluctuation (4°C) up to a maximum of 80% at a 14°C amplitude, whether the seeds were stratified or not. A period of cold stratification reduced primary dormancy, and germination was always lower (<25%) in the dark (constant), irrespective of temperature fluctuation regimes (Brändel, 2006). After-ripening effects on germination rates of seeds of *S. palustris* were not detected, and germination rates of seeds collected in autumn and stored at room temperature for 7 months did not increase (Brändel, 2006). Investigations on *S. germanica* L. seeds (Güteryüz et al., 2011) showed that treatments with GA or a combination of GA and KIN were successful in terminating seed dormancy. Seeds treated with a combination of 250 ppm gibberellic acid (GA) + 50 ppm kinetin (KIN) after 30 days of stratification (wet chilling + 4°C) achieved the highest germination rate (95%) under continuous darkness and photoperiodic (12 h dark/20°C and 12 h light/10°C) conditions. However, without stratification in cold wet sand, only slightly more than a third of seeds treated with GA germinated. In another experiment without pre-treatment for 26 days, *S. germanica* seeds germinated neither in light nor in darkness at temperatures between 5 and 26°C. Even at higher temperatures (29–35°C), a low rate of germination was observed (Dunn, 1997). However, seed germination of *S. byzantina* K. Koch was not markedly stimulated by GA concentrations in both light/dark and complete darkness conditions (Rezvani et al., 2021). In laboratory experiments, Ismaili et al. (2023) showed that alternating temperatures strongly affected the seed germination capacity and speed in the case of *S. mouretii* Batt. & Pit. The temperature regimes of 25/10°C and 35/20°C significantly increased the germination percentage to 66.5% and 65.5%, respectively. Furthermore, the most effective pre-treatment improving germination was GA at an optimal concentration of 500 mg/l by improving the germination percentage to 91.5% and reducing the median time of germination (T_{50}) and mean germination time (MGT) to 11.7 days and 9.9 days, respectively.

The timing of germination is a critical circumstance in a plant's life cycle (Donohue, 2005), which is determined by the seed dormancy mechanisms/behaviour (Postma & Ågren, 2015). From an ecological perspective, seed dormancy can be defined as the prevention of germination even when suitable conditions are prevalent (Benech-Arnold et al., 2000). The dormancy mechanism allows a species to synchronise its germination with favourable environmental conditions, which increases its probability of survival and establishment (Baskin & Baskin, 2014). In nature, based on its timing, two principal categories of seed dormancy are distinguished. Primary dormancy is an innate seed characteristic that establishes during seed maturation on the mother plant, while secondary dormancy occurs after shedding from the parent plant in non-dormant seeds that were exposed to abiotic stresses associated with prolonged lack of external environmental conditions required for germination (e.g. temperature, light, soil moisture, oxygen, nitrate) (Buijs, 2020). The causes of primary seed dormancy are complex; it may be developed in the embryo or imposed by the tissues that surround the embryo. In several species, both the embryo and the tissues enclosing it impose dormancy (Hilhorst et al., 2010). In temperate regions, the seeds of most plant families exhibit physiological dormancy, which is caused by insufficient embryo growth potential (Baskin & Baskin, 2014). Depending on its depth, several techniques can be used to overcome seed dormancy. These techniques include, for example, treatments with GA, potassium nitrate (KNO₃), cold and warm stratification, physical and chemical scarification, and leaching (Baskin & Baskin, 2014). The stratification treatments mimic the slow seasonal changes that modify seed dormancy levels gradually over time (mean temperature, seed moisture), while GA addition as well as light and temperature fluctuations are signals that may indicate a “spatial window” of opportunity for germination, with a sudden termination of seed dormancy (Benech-Arnold et al., 2000; Brändel, 2006; Ismaili et al., 2023).

We hypothesized that the combination of stratification and gibberellic acid would release the dormancy of *S. annua* seeds. To explore this hypothesis, we addressed the

following main aims: (I) exploration of the effect of different moist stratification regimes for 4 weeks (in cold/warm sand) on breaking dormancy and promoting germination, and (II) determination of the effect of gibberellic acid on seed germination under two light-temperature regimes (constant/fluctuating) for *S. annua*. Controlled laboratory experiments were conducted to achieve these objectives, preceded by stratification pre-treatments simulating natural environmental conditions. Identifying effective methods for breaking the dormancy of *S. annua* seeds in laboratory conditions may help to achieve higher and more uniform germination rates. The availability of such techniques could be an important first step towards introducing *S. annua* into cultivation.

2. Materials and methods

2.1. Collection and storage of seeds

The seeds of *S. annua* were manually harvested from natural matured plants in a population growing in a stubble of an extensively managed phacelia (*Phacelia tanacetifolia* Benth.) field in the Lesser Plain of north-western Hungary (47°84'97"N; 17°09'47"E). This specific phacelia field was cultivated for seed production, which is quite common in the region (Dunai & Pinke, 2023), and it was left uncultivated after harvest to preserve its spontaneously emerging *S. annua* population that was already known to local beekeepers (Pinke et al., 2020). The gathering of *S. annua* seeds took place in the middle of September 2020. The seeds were cleaned and stored dry in paper bags in ambient conditions (20±5°C) in the laboratory until the start of the experiments, which began on 10 November 2020.

2.2. Germination experiments

2.2.1. Dormancy breaking methods

We analyzed the impact of three possible treatments on the germination of *S. annua* in a full factorial design. The first one was a *stratification* (STR) treatment implemented at different levels: no stratification (NS: using fresh seeds as the control), cold stratification (CS: 5°C for 4 weeks), and warm stratification (WS: 21°C for 4 weeks). The second treatment involved the application of *gibberellic acid* (GA) with two possible levels: no GA (DW: distilled water as the control) and gibberellic acid (GA: 250 mg/l solution of GA). The third treatment was an environmental *light-temperature regime* (LTR) treatment, with two possible levels: a “constant regime” (CR: continuous darkness at a stable 20°C temperature as the control) and a “fluctuating regime” (FR: 14 h at 20°C & 300 μmol photons m⁻² s⁻¹ fluorescent light, followed by 10 h at 10°C in darkness to simulate a germination-friendly diurnal light-temperature pattern).

2.2.2. Germination test protocol

We performed five replications for treatment combinations with no stratification (NS) and four replications for combinations involving stratified seeds (CS, WS). The stratification treatment was implemented first: the seeds selected for CS or WS were wrapped in fine nylon mesh, placed in moist sand-filled containers, and exposed to the selected temperature for four weeks. The germination trials were performed after the (optional) stratification in incubation chambers. At the beginning of each trial, seeds were tested for viability using a 1% solution of 2,3,5-triphenyl-tetrazolium chloride (TTC) with 50 seeds in four replications (Peters, 2000). Selected *S. annua* seeds were washed under running tap water, disinfected with a 50% ethanol solution for 4 minutes, and then rinsed 5–6 times with sterile deionized water. After rinsing, the seeds were not dried but immediately placed in incubation chambers. In each trial, 50 seeds were placed on two layers of filter paper (MN 640 W) in a Petri dish (with 9 cm diameter) and were moistened with 6 ml of the selected solution (DW or GA). The Petri dishes were sealed with two layers of para-film to prevent moisture loss and, in the constant dark regime (CR), they were also covered with two layers of aluminium foil to avoid light penetration. The number of germinated seeds was recorded

2, 7, and 15 days after starting the germination test, and the accumulated germination percentage (AGP) after the 15 days was used as the response variable. Seeds were considered to be germinated when they produced a visible radicle longer than 1 mm (ISTA, 1985).

2.3. Statistical analysis

To account for the binary nature of germination data, we applied a logistic regression model with the logit link function for the data analysis. To evaluate the effects and interactions of the experimental factors, a series of nested models were fitted and compared using Likelihood Ratio Tests (LRT) to assess the significance of main effects (STR, GA, LTR) and their interactions (STR × GA, STR × LTR, GA × LTR, and finally STR × GA × LTR). Results were considered statistically significant at a 95% confidence interval ($p < 0.05$ and $p < 0.001$). The entire analysis was performed in R (version 4.3.2.) (R Core Team, 2024).

3. Results

3.1. General traits of seed dormancy release and germination

According to the tetrazolium test, 90% of the seeds of *S. annua* were viable. The accumulated germination percentage (AGP) in the control trials (i.e. NS, DW, CR) was 2.4%, indicating that the vast majority of freshly matured (NS) seeds were in the state of primary dormancy (Figure 1).

3.2. Effect of stratification on seed dormancy release and germination

The results indicate that stratification (STR) had a strongly significant effect on the germination of *S. annua* seeds (Table 1). Nevertheless, despite this significant overall impact, the effect of stratification (STR) was clearly different in the various treatment combinations. With the control levels of the two other treatments, neither cold (CS) nor warm stratification (WS) had a clear effect (AGP = 0 – 3%) (Figure 1). However, warm stratification (WS) combined with gibberellic acid (GA) significantly increased the germination percentage, whereas cold stratification (CS) with GA showed no similar effect (Figure 1).

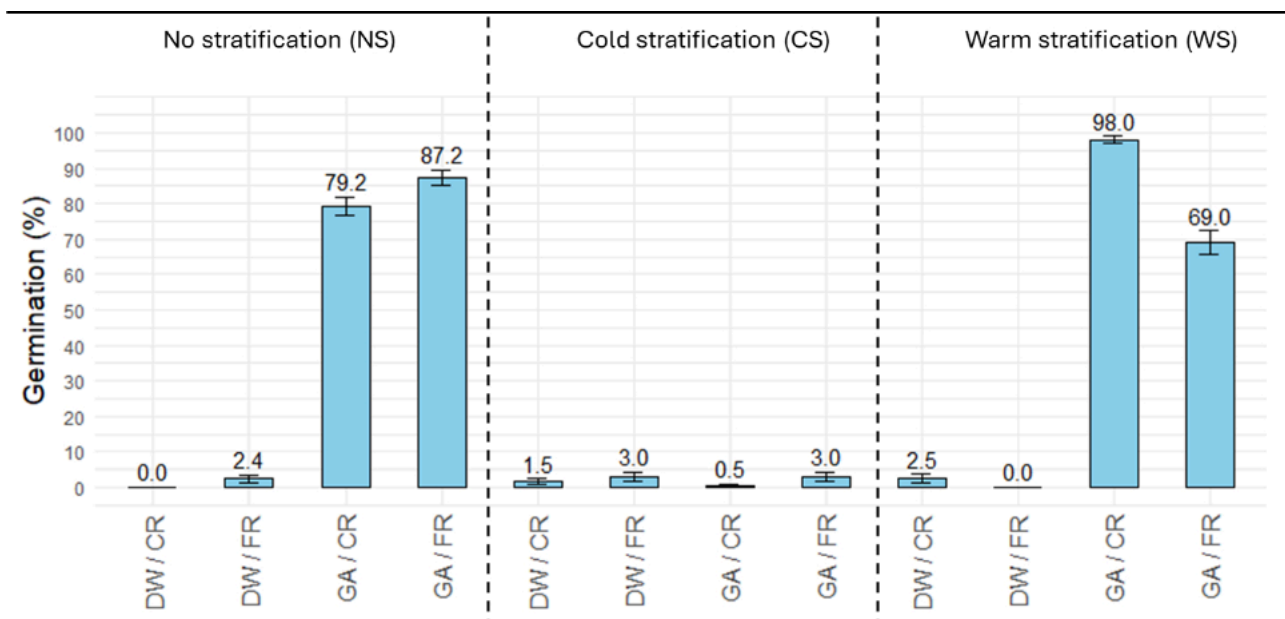


Figure 1 Effects of stratification, gibberellic acid, and light-temperature regimes on dormancy release and germination of *Stachys annua* seeds. Note: DW: distilled water; GA: 250 mg/l solution of GA; CR: constant regime (20°C in continuous darkness); FR: fluctuating regime (simulated diurnal light and temperature changes).

Table 1 Results of the LRT test

Treatments and treatment combinations	df	LRT	p-value
Stratification (STR)	2	810.3	< 2e-16***
Gibberellic acid (GA)	1	1446.7	< 2e-16***
Light-temperature regime (LTR)	1	5.09	0.024*
STR x GA interaction	2	84.99	< 2e-16***
STR x LTR interaction	2	82.67	< 2e-16***
GA x LTR interaction	1	0.52	0.473 ^{ns}
Three-way interaction	2	5.86	0.053 ^{ns}

Note: df – degree of freedom; LRT – likelihood ratio test; ^{ns} – not significant; * – significance at $p < 0.05$ level; and *** – significance at $p < 0.001$ level.

3.3. Effect of gibberellic acid on seed dormancy release and germination

Gibberellic acid (GA) also had a highly significant overall influence on the seed germination (Table 1). The presence of GA significantly increased the germination of non-stratified (NS) and warm stratified (WS) seeds under both light-temperature regimes (LTR). In fact, these trials showed an enormous impact of gibberellic acid (GA), changing germination rates from close to zero to very high (70–100%), with an almost unbeatable 98% rate in one of the treatment combinations (WS/GA/CR, Figure 1). In contrast, cold stratified seeds (CS) that had been stored in wet sand at low temperature for 4 weeks (cold stratification) failed to germinate even with GA treatment (AGP = 0.5 and 3%). These results suggest that cold stratification (CS) has increased the seed dormancy level compared to freshly matured seed lots (NS) treated with the GA solution (Figure 1).

3.4. Effect of light-temperature regimes on seed dormancy release and germination

From the three factors studied, light-temperature regimes (LTR) seemed to exert the weakest impact on the germination process. Nevertheless, the interaction of stratification (STR) with the environmental regime (STR × LTR) was found clearly significant (Table 1). This is probably the consequence of LTR having the opposite effect for warm stratified seeds (WS) than for the other two types of stratification. While for no stratification (NS) and cold stratification (CS) the fluctuating regimes (FR) resulted in slightly higher germination, after warm stratification (WS) the constant regime (CR) seemed to provide a better germination support, including the close to unbeatable 98% results (Figure 1).

4. Discussion

It is well established from the relevant literature that freshly harvested, mature seeds of members of the *Stachys* genus are generally in primary dormancy (Dunn, 1997; Godefroid et al., 2010; Graae et al., 2009; Güleriyüz et al., 2011; Pinfield et al., 1972, 1975). The results of this study demonstrate that most freshly harvested mature seeds of *S. annua* were dormant at maturity. Seeds failed to germinate in response to short-time (4 weeks) cold or warm stratification treatments with distilled water in both constant and fluctuating light-temperature conditions. A similar phenomenon was also observed in the study on seeds of *S. germanica* (Güleriyüz et al., 2011). According to Pinfield et al. (1972), *S. alpina* seeds did not germinate after less than 4 weeks of cold chilling, and only a limited increase in germination (20–30%) occurred even after periods as long as 12–20 weeks (Pinfield et al., 1975). The study of Graae et al. (2009) indicated that fresh seeds of *S. sylvatica*, gathered in various habitats across central and north-western Europe, had very poor germination (0–19%). After 18 weeks

of cold stratification followed by 6 weeks of warm incubation (14 h light at 20°C and 10 h dark at 10°C), germination fluctuated between 13% and 54%. When seeds were subjected first to 6 weeks of warm incubation followed by 18 weeks of cold incubation and finally 6 weeks of warm incubation, germination was raised to 50–91%. This means that in both natural habitats and agroecosystems, the dormancy of *S. annua* seeds presumably takes extended periods of time to disappear. This is confirmed by its prolonged field emergence, which usually takes place only in the second spring after the maturation (Dunai et al., 2020).

Based on our results, it appears that the seeds of *S. annua* are in non-deep physiological dormancy, with gibberellic acid playing a substantial role in terminating it. The application of GA alone considerably improves the germination of freshly harvested mature *S. annua* seeds. Similar results were found by Pinfield et al. (1972) for *S. alpina*, where maximum germination (> 90%) was achieved at 104 mg/l of GA. Ismaili et al. (Ismaili et al., 2023) reported that the most efficient pre-treatment to boost seed germination in *S. mouretii* was GA at an optimum concentration of 500 mg/l, increasing the germination rate up to 91.5%. Nevertheless, the environmental factors required for gradually overcoming seed dormancy act by increasing the active level of endogenous GA, so externally applied GA often substitutes for certain environmental stimuli such as specific temperature pre-treatment (stratification) or light (Karam & Al-Salem, 2001; Karssen & Laçka, 1986). This was confirmed by the results of our experiment with freshly collected mature *S. annua* seeds. At the same time, in addition to the substitution effect, the simultaneous combination of GA with other seed dormancy release methods, such as cold stratification (Balouchi & Sanavy, 2006) or other chemicals, such as potassium nitrate (KNO₃) (Mousavi et al., 2019), and plant hormones, such as cytokinin (Ganjali et al., 2022), can have a synergistic effect in terminating the dormancy of the treated seed. Our results suggest that the highest germination rate (98%) can be reached in the seeds handled with a combination of 250 mg/l GA and 4 weeks of warm stratification, when germination takes place in the dark for 24 hours at 20°C. Comparing these results with laboratory studies with other species of the genus *Stachys*, it seems that the germination biology of *S. annua* exhibits strong similarities with the germination biology of *S. alpina*. To obtain this among natural circumstances, longer periods of seeds remaining on the mother plant and lying in the soil are needed.

At the same time, our findings do not exclude the probability that, beside the high ABA/GA ratio in tissues during seed maturation, the dormancy of *S. annua* seeds may also be influenced by inhibitors in the embryo and/or by the immaturity of the embryo itself. However, to clarify this, further investigations are required. In addition, it should also be noted that the type of pollination may also influence the different germination responses of various populations of *Stachys* species. Wilcock and Jones (1974) observed that mature seeds from plants collected from cross-pollinated stands of *S. palustris* showed nearly twice the germination rate (90%) of their peers collected from self-pollinating plants (52%). We have not performed such analyses on *S. annua* seeds, but considering that the site of the seed collection was frequently visited both by honey bees and wild pollinators (Pinke et al., 2020), the high germination rates of our seed samples suggest that they originated mainly from cross-pollinated plants. Hegi (1975) also documented that *S. annua* is primarily an insect-pollinated species, though self-pollination can occasionally also occur.

In our experimental design, we analyzed the effect of light and temperature regimes jointly as a combined treatment. This combined analysis was partly inspired by nature (where light and temperature fluctuations also occur somewhat jointly: deeply buried seeds receive neither, while seeds on the surface experience both), and partly necessitated by the availability of technical equipment in our laboratory (a limited number of incubators). Of course, this way we cannot know where the effects of “LTR” (our combined light-temperature regime treatment) come from: are these effects primarily caused by the changing temperature, or should they rather be attributed to the presence of (or changes in) light? Answering this question would demand a follow-up experiment. Since LTR had only minor impacts on the germination rate and (in particular) the most promising treatment combination involved a stable environmental regime, this research direction might not be urgent from a practical perspective.

Nevertheless, several further challenges still need to be solved before *S. annua* can become a successful crop. This list includes several complex technological and even social challenges ranging from motivating farmers for leaving their stubbles unploughed to developing efficient techniques for the laborious steps of seed harvesting (Karssen & Lačka, 1986; Pinke et al., 2021). In any case, our results are very encouraging for the development of a dormancy-breaking technology and its large-scale application on the seeds of *S. annua* to achieve a uniform germination which will enable its future growing as a valuable bee pasture.

5. Conclusions

Our results confirmed our hypothesis that the combination of stratification and gibberellic acid would release the dormancy of *S. annua* seeds. Our findings proved that gibberellic acid plays an important role in breaking the seed dormancy of *S. annua* and can provide a replacement for the stratification requirement of seeds of this species for germination, which can be used to produce uniformly germinating seeds for commercial purposes. The seed dormancy-breaking treatments should be performed in strictly controlled conditions by seed companies. The cost of GA should be incorporated into the price of the seeds, ensuring that the final cost remains manageable for beekeepers and farmers.

Availability of data and materials

The raw data is available upon request from the corresponding author.

References

- Abbasi, M. R. (2020). Breaking seed dormancy in some Iranian wild clovers (*Trifolium* spp.) selected as promising new germplasm. *Genetic Resources and Crop Evolution*, 67(6), 1577–1585. <https://doi.org/10.1007/s10722-020-00933-4>
- Andriamparany, J. N., & Buerkert, A. (2019). Effect of ultrasonic dormancy breaking on seed germination and seedling growth of three wild yam species (*Dioscorea* spp.) from SW-Madagascar. *Genetic Resources and Crop Evolution*, 66(6), 1167–1174. <https://doi.org/10.1007/s10722-019-00779-5>
- Balouchi, H. R., & Sanavy, S. A. M. M. (2006). Effect of gibberellic acid, prechilling, sulfuric acid and potassium nitrate on seed germination and dormancy of annual Medics. *Pakistan Journal of Biological Sciences*, 9(15), 2875–2880. <https://doi.org/10.3923/pjbs.2006.2875.2880>
- Baskin, C. C., & Baskin, J. M. (2014). Seeds: Ecology, biogeography, and evolution of dormancy and germination. In *Seeds: Ecology, Biogeography, and Evolution of Dormancy and Germination* (p. 1586). Elsevier Inc. <https://doi.org/10.1016/C2013-0-00597-X>
- Benech-Arnold, R. L., Sánchez, R. A., Forcella, F., Kruk, B. C., & Ghera, C. M. (2000). Environmental control of dormancy in weed seed banks in soil. *Field Crops Research*, 67(2), 105–122. [https://doi.org/10.1016/S0378-4290\(00\)00087-3](https://doi.org/10.1016/S0378-4290(00)00087-3)
- Berkó, J., & Kardos, R. (1937). Rendellenes összetételű mézek. [Honeys with unusual content]. *Mezőgazdasági Kutatások*, 10, 177–180.
- 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(1), 11–28. <https://doi.org/10.1007/s11273-005-1548-5>
- Buijs, G. (2020). A perspective on secondary seed dormancy in *Arabidopsis thaliana*. *Plants*, 9(6), 1–9. <https://doi.org/10.3390/plants9060749>
- Csikó, J., Balogh, L., Dancza, I., Gyulai, F., Jakab, G., Király, G., Lehoczy, É., Mesterházy, A., Pósa, P., & Wirth, T. (2023). Checklist of alien vascular plants of Hungary and their invasion biological characteristics. *Acta Botanica Hungarica*, 65(1–2), 53–72. <https://doi.org/10.1556/034.65.2023.1-2.3>
- Cuena Lombraña, A., Dessì, L., Podda, L., Fois, M., Luna, B., Porceddu, M., & Bacchetta, G. (2024). The effect of heat shock on seed dormancy release and germination in two rare and endangered *Astragalus* L. species (Fabaceae). *Plants*, 13(4), 484. <https://doi.org/10.3390/plants13040484>
- Donohue, K. (2005). Seeds and seasons: Interpreting germination timing in the field. *Seed Science Research*, 15(3), 175–187. Cambridge Core. <https://doi.org/10.1079/SSR2005208>
- Dunai, É., Pinke, G., Magyar, L., Kulmány, I., Szűcs, Z., & Roszík, P. (2020). Tarlóvirágmag begyűjtése és tisztítása méhlegelők vetéséhez. *Biokultúra*, 31(6), 18–21. [Collecting and cleaning the seeds of *Stachys annua* for sowing bee pastures].

- Dunai, É., & Pinke, G. (2023). Historical review of lacy phacelia (*Phacelia tanacetifolia* Benth.) cultivation based on Hungarian botanical, apicultural and agronomical studies. *Botanikai Közlemények*, 110(1), 43–60. <https://doi.org/10.17716/BotKozlem.2023.110.1.43>
- Dunn, A. J. (1997). *Stachys germanica* L. *Journal of Ecology*, 85(4), 531–539. <https://doi.org/10.2307/2960575>
- Ekren, S., Gökçöl, A., Yıldırım Keskinoglu, A., & Paylan, İ. C. (2024). Optimizing germination performance of Lamiaceae family seeds: Insights from research. *Journal of Plant Diseases and Protection*, 131(3), 999–1008. <https://doi.org/10.1007/s41348-023-00857-y>
- Fuller, D. Q., & Allaby, R. (2018). Seed Dispersal and Crop Domestication: Shattering, Germination and Seasonality in Evolution Under Cultivation. In *Annual Plant Reviews online* (pp. 238–295). <https://doi.org/10.1002/9781119312994.apr0414>
- Ganjali, S., Khajeh, H., Gholami, Z., Jomeh-Ghasemabadi, Z., & Fazeli-Nasab, B. (2022). Evaluation of Dormancy Failure *Datura stramonium* Plant Seeds under the Influence of Different Treatments. *Agrotechniques in Industrial Crops*, 2, 32–41. <https://doi.org/10.22126/ATIC.2022.7656.1049>
- Godefroid, S., van de Vyver, A., & Vanderborght, T. (2010). Germination capacity and viability of threatened species collections in seed banks. *Biodiversity and Conservation*, 19(5), 1365–1383. <https://doi.org/10.1007/s10531-009-9767-3>
- Graae, B. J., Verheyen, K., Kolb, A., Van Der Veken, S., Heinken, T., Chabrierie, O., Diekmann, M., Valtinat, K., Zindel, R., Karlsson, E., Ström, L., Decocq, G., Hermy, M., & Baskin, C. C. (2009). Germination requirements and seed mass of slow- and fast- colonizing temperate forest herbs along a latitudinal gradient. *Écoscience*, 16(2), 248–257. <https://doi.org/10.2980/16-2-3234>
- Güleryüz, G., Kirmizi, S., Arslan, H., & Sakar, F. S. (2011). Dormancy and germination in *Stachys germanica* L. subsp. *Bithynica* (Boiss.) Bhattacharjee seeds: Effects of short-time moist chilling and plant growth regulators. *Flora*, 206(11), 943–948. <https://doi.org/10.1016/j.flora.2011.07.003>
- Hammer, K. (1984). Das Domestikationssyndrom. [The domestication syndrome]. *Die Kulturpflanze*, 32(1), 11–34. <https://doi.org/10.1007/BF02098682>
- Hegi, G. (1975). *Illustrierte Flora von Mittel-Europa V/4*. – Verlag Paul Parey, Berlin, Hamburg [Illustrated Flora of Central Europe V/4].
- Hilhorst, H. W. M., Finch-Savage, W. E., Buitink, J., Bolingue, W., & Leubner-Metzger, G. (2010). Dormancy in Plant Seeds. In E. Lubzens, J. Cerda, & M. Clark (Eds.), *Dormancy and Resistance in Harsh Environments* (pp. 43–67). Springer Berlin Heidelberg. https://doi.org/10.1007/978-3-642-12422-8_4
- Ismaili, S. E., Maurady, A., Lachkar, M., Britel, M. R., & Bakali, A. H. (2023). Effect of temperature and different pre-treatments on seed germination of *Stachys mouretii* Batt. & Pit. *Journal of Applied Research on Medicinal and Aromatic Plants*, 32. <https://doi.org/10.1016/j.jarmap.2022.100438>
- ISTA (1985). International rules for seed testing. *Seed Science Technology*, 13(2), 307–513.
- Juhász, J. (1983). A tarlóvirág vagy tisztesfű. [Yellow-woundwort or annual hedgenettle]. *Magyar Szó*, 40 (156), 18.
- Karam, N. S., & Al-Salem, M. M. (2001). Breaking dormancy in *Arbutus andrachne* L. seeds by stratification and gibberellic acid. *Seed Science and Technology*, 29(1), 51–56.
- Karssen, C. M., & Laçka, E. (1986). A Revision of the Hormone Balance Theory of Seed Dormancy: Studies on Gibberellin and/or Abscisic Acid-Deficient Mutants of *Arabidopsis thaliana*. In M. Bopp (Ed.), *Plant Growth Substances 1985* (pp. 315–323). Springer Berlin Heidelberg.
- Khan, M. H., Dar, N. A., Alie, B. A., Mir, G. H., Fayaz, U., Khan, A., Bashir, B., Ahmad, A., Mansoor, S., Chung, Y. S., & Heo, S. (2023). Plant Growth Hormones and Micro-Tuberization in Breaking the Seed Dormancy of *Bunium persicum* (Boiss.) Fedts. *Plants*, 12(17), 3163. <https://doi.org/10.3390/plants12173163>
- Kirmizi, S. (2023). Effects of pre-treatments on seed dormancy and germination of endemic *Muscari bourgaei* Baker. *Agronomy*, 13(9), 2438. <https://doi.org/10.3390/agronomy13092438>
- Kołodziejek, J. (2018). Seed dormancy in cereal weed *Adonis flammula* Jacq. (Ranunculaceae). *Journal of Agricultural Science and Technology*, 20, 109–120.
- Krasnylenko, Y., Hýlová, A., Sosnovsky, Y., Ulbrichová, M., Spíchal, L., & Piwowarczyk, R. (2023). Seed germination of *Cistanche armena* (Orobanchaceae), a rare endangered holoparasitic species endemic to Armenia. *Seed Science Research*, 33(2), 118–125. <https://doi.org/10.1017/S0960258523000090>
- Mousavi, L., Ishak, W. R. W., & Mousavi, M. (2019). Evaluation of physicochemical methods for dormancy breakage and germination of *Datura stramonium* seeds. *Journal of Chemical Health Risks*, 9(3), 217–224. <https://doi.org/10.22034/jchr.2019.668186>

- Peters, J. (2000). *Tetrazolium Testing Handbook, Contribution No. 29*. Association of Official Seed Analysis.
- Pinfield, N. J., Martin, M. H., & Stobart, A. K. (1972). The control of germination in *Stachys alpina* (L.). *New Phytologist*, 71(1), 99–104. <https://doi.org/10.1111/j.1469-8137.1972.tb04815.x>
- Pinfield, N. J., Martin, M. H., & Stobart, A. K. (1975). Hormonal changes during after-ripening in seeds of *Stachys alpina* L. *New Phytologist*, 75(1), 81–85. <https://doi.org/10.1111/j.1469-8137.1975.tb01373.x>
- Pinke, G. (2020). The status of arable plant habitats in Eastern Europe. In C. Hurford, P. Wilson, & J. Storkey (Eds.), *The changing status of arable habitats in Europe* (pp. 75–87). Springer. https://doi.org/10.1007/978-3-030-59875-4_6
- Pinke, G., Dunai, É., & Czucz, B. (2021). Rise and fall of *Stachys annua* (L.) L. In the Carpathian Basin: A historical review and prospects for its revival. *Genetic Resources and Crop Evolution*, 68(7), 3039–3053. <https://doi.org/10.1007/s10722-021-01219-z>
- Pinke, G., Dunai, É., Vona, V., Varga, T., & Zsuppán, L. (2020). Tisztesfüves facéliatarló megőrzése méhlegelőnek. [Preserving a phacelia stubble with *Stachys annua* for bee pasture]. *Méhészújság*, 7(11), 16–18.
- Pinke, G., & Pál, R. (2009). Floristic composition and conservation value of the stubble-field weed community, dominated by *Stachys annua* in western Hungary. *Biologia*, 64(2), 279–291. <https://doi.org/10.2478/s11756-009-0035-5>
- Postma, F. M., & Ågren, J. (2015). Maternal environment affects the genetic basis of seed dormancy in *Arabidopsis thaliana*. *Molecular Ecology*, 24(4), 785–797. <https://doi.org/10.1111/mec.13061>
- R Core Team (2024). *R: A language and environment for statistical computing*. – R Foundation for Statistical Computing. [Computer software]. URL: www.r-project.org.
- Rezvani, M., Amini, B. J., & NikkhhahKouchaksaraei, 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>
- Santiago, A., Herranz, J. M., & Ferrandis, P. (2023). Seed dormancy break and germination by a rare relict of the Würmian glaciation in the Iberian Peninsula: *Euonymus latifolius* (Celastraceae). *Seed Science Research*, 33(2), 101–106. <https://doi.org/10.1017/S0960258523000132>
- Singh, N., Gore, P. G., & Aravind, J. (2020). Breaking seed coat impermeability to aid conservation and utilization of wild *Vigna* species. *Genetic Resources and Crop Evolution*, 67(2), 523–529. <https://doi.org/10.1007/s10722-019-00872-9>
- Skrajna, T., Kubicka, H., & Gozdowski, D. (2018). Phenotypic and genotypic variation of declining segetal flora: The case of *Stachys annua* (Lamiaceae) populations established in North-Eastern Poland. *Polish Journal of Ecology*, 66(1), 23–35. <https://doi.org/10.3161/15052249PJE2018.66.1.003>
- Slade, E. A., & Causton, D. R. (1979). The germination of some woodland herbaceous species under laboratory conditions: A multifactorial study. *New Phytologist*, 83(2), 549–557. <https://doi.org/10.1111/j.1469-8137.1979.tb07476.x>
- Spengler, R. N., III. (2020). Anthropogenic Seed Dispersal: Rethinking the Origins of Plant Domestication. *Trends in Plant Science*, 25(4), 340–348. <https://doi.org/10.1016/j.tplants.2020.01.005>
- Thompson, K. & Grime, J. P. (1979). Seasonal variation in the seed banks of herbaceous species in ten contrasting habitats. *Journal of Ecology*, 67, 893–921. <https://doi.org/10.2307/2259220>
- Wilcock, C. C. & Jones, B. M. G. (1974). The identification and origin of *Stachys x ambigua* Sm. *Watsonia*, 10, 139–147.
- Yalamalle, V. R., Dunna, V., Chawla, G., Mishra, G. P., P., V. H., Ahmad, D., Lal, S. K., Joshi, D. C., & Meena, R. P. (2024). Overcoming physiological dormancy in common buckwheat (*Fagopyrum esculentum* Moench). *Genetic Resources and Crop Evolution*, 71(5), 1659–1672. <https://doi.org/10.1007/s10722-023-01729-y>
- Zhao, X., Wang, F., Wang, L., Wang, Q., Liu, A., & Li, Y. (2024). Deep simple epicotyl morphophysiological dormancy in seeds of endemic Chinese *Helleborus thibetanus*. *Agriculture*, 14(7), 1041. <https://doi.org/10.3390/agriculture14071041>