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Gibberellic acid priming enhances *Zostera marina* seed germination: implications for restoration

Riccardo Pieraccini[1,2]*, Grace Dixon[3], Tobias Dolch[4], Nico Koedam[2], Sarah Merolla[3], Lisa Picatto[1], Francesca Petrucci[5], Mirta Teichberg[3], Tom Van der Stocken[2] and Ann Vanreusel[1]

[1]Marine Biology Research Group, Department of Biology, Ghent University, Ghent, Belgium, [2]Ecology, Evolution and Genetics Research Group (bDIV), Biology Department, Vrije Universiteit Brussel, Brussels, Belgium, [3]The Ecosystems Center, Marine Biological Laboratory, Woods Hole, MA, United States, [4]Coastal Ecology, Alfred-Wegener-Institut Helmholtz-Zentrum für Polar- und Meeresforschung – Wattenmeerstation Sylt, Sylt, Germany, [5]Phycology Research Group, Department of Biology, Ghent University, Ghent, Belgium

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EDITED BY
Elisabeth Marijke Anne Strain,
University of Tasmania, Australia

REVIEWED BY
W. Judson Kenworthy,
Independent Researcher, United States
Shaochun Xu,
Chinese Academy of Sciences (CAS), China
Elisa Petrussa,
University of Udine, Italy

*CORRESPONDENCE
Riccardo Pieraccini
riccardo.pieraccini@ugent.be

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Eelgrass (*Zostera marina*) meadows are foundational marine habitats that support biodiversity and provide key ecosystem services, including carbon sequestration, fisheries production, and shoreline stabilization. Yet, their global decline demands expanded restoration efforts. Seed-based restoration offers a scalable alternative to labor-intensive transplantation, but low germination and seedling emergence remain major constraints. Here, we tested gibberellic acid (GA_3) priming on dormant *Z. marina* seeds from three temperate North Atlantic populations (one intertidal annual population and two subtidal perennial populations) differing in origin, age, and handling history. GA_3 priming consistently improved germination success and reduced time to germination across all populations, with optimal responses at 9 and 20 mg L^{-1}. Positive effects in the older seed cohort (stored for approximately 16 months) and in sterilized seeds suggest that GA_3 can mitigate physiological constraints associated with long-term storage or handling. Although the experimental design does not separate ecological from geographic influences, including populations from contrasting habitats provides a valuable test of GA_3 priming under varied seed conditions. As a simple, transferable pre-treatment, GA_3 priming shows strong potential to enhance seed readiness and increase nursery or restoration seed stocks, supporting more sustainable use of limited *Z. marina* seed resources. We further outline how GA_3 priming can be incorporated as a short immersion step within existing seed-handling workflows and provide a back-of-the-envelope consumables cost estimate to contextualize scale-up potential.

KEYWORDS

dormancy alleviation, gibberellic acid priming, seagrass restoration, seed biology and germination, seed-based restoration

1 Introduction

Seagrass meadows are increasingly recognized as critical coastal habitats, providing major ecological (Dewsbury et al., 2016; Nordlund et al., 2018) and societal benefits (Foster et al., 2025). They contribute to carbon sequestration, stabilize sediments (Miyajima et al., 2025), and provide essential nursery grounds that support coastal biodiversity and human well-being (Cullen-Unsworth et al., 2014; Whitfield, 2017). Yet, global declines of seagrass meadows have been widely reported (Waycott et al., 2009; Dunic et al., 2021; Turschwell et al., 2021), rendering their restoration an urgent conservation priority.

Seagrass protection and conservation remain essential for limiting habitat loss and fragmentation (Coals et al., 2025a; Duarte et al., 2025). Passive restoration, defined as removing or reducing stressors to enable natural recovery (e.g. improving water quality and mitigating local impacts), can complement protection and conservation efforts but cannot, by itself, reverse decades of degradation unless sufficiently large source seagrass populations remain to supply propagules for recolonization (Hemraj et al., 2024). Active restoration is therefore expanding rapidly to accelerate recovery and meet ambitious international targets (United Nations Environment Programme, 2020; Council of the European Union & European Parliament, 2024). Although large-scale restoration projects have demonstrated successful outcomes when ideal conditions align, failure rates remain high (Van Katwijk et al., 2016).

Seed-based restoration has emerged as a conceptually scalable, and relatively low-cost alternative to shoot transplantation, enabling deployment over large areas with lower labor requirements while maintaining genetic diversity (Unsworth et al., 2019; Tan et al., 2023; Ortiz et al., 2025). Pioneering large-scale seed broadcasting in the Virginia Coastal Bays (Orth et al., 2020) demonstrated the feasibility of restoring extensive eelgrass meadows through direct seeding. Building on this foundation, recent sowing tool developments, such as Dispenser Injection Systems (DIS) (Govers et al., 2022), hessian seed bags (Unsworth et al., 2019), and autonomous seeding robots (Robocean, n.d.; Ulysses, n.d.), and large-scale seed-ball burial (> 1, 700 ha) in northern China (Liu et al., 2022), are making landscape-scale seed-based restoration increasingly feasible (Gräfnings et al., 2023a). Since natural seed banks remain the main source of propagules, and completing the full seed-to-seed cycle in nurseries is still technically challenging despite recent advances (Tanner and Parham, 2010; de Tourtoulon-Adams, 2022; Unsworth et al., 2023; Great Barrier Reef Foundation, 2024), maximizing the potential of available seed stocks, without compromising vulnerable donor populations, remains a key priority. By enhancing germination success through GA_3 priming, this study contributes to more efficient use of limited seed stock and reduces harvesting pressure on natural donor meadows. This is especially relevant in Europe, where widespread habitat degradation limits the availability of sustainable seed sourcing for restoration (de los Santos et al., 2019; Nordlund et al., 2024).

Zostera marina, the most widely distributed seagrass species in the Northern Hemisphere, is also the most commonly used seagrass species in seed-based restoration due to its large seed production and broad geographic distribution. This species reproduces both sexually, through seeds, and clonally, through rhizome expansion, with the relative contribution of each strategy varying across ecotypes and environments (Jackson et al., 2017; Guerrero-Meseguer et al., 2022). Annual populations depend almost entirely on sexual reproduction and typically allocate most resources to seed production, whereas subtidal perennial populations rely on a combination of both strategies, with clonal propagation often predominant for long-term meadow persistence. The species forms a transient seed bank, as most viable seeds germinate within a single season and rarely persist beyond several overwintering periods (Jarvis and Moore, 2010; Jarvis et al., 2014; Baker et al., 2024). Yet, germination and seedling emergence often remain below 5% (Unsworth et al., 2023), far below the levels required to support restoration at an effective scale. Despite considerable effort, the drivers causing low success rates remain poorly understood. Both intrinsic and extrinsic factors contribute to the low germination and seedling emergence observed in *Z. marina*. Intrinsic mechanisms, such as seed dormancy regulated by environmental cues and storage conditions, interact with extrinsic factors related to seed collection, handling, and restoration practices (Brodersen and Pedersen, 2024; Lowell et al., 2024; Pieraccini et al., 2025a; Sgambelluri et al., 2025; Takeda et al., 2025; Zhang et al., 2025). Identifying reliable methods to improve germination outcomes, therefore, is critical for enhancing both the ecological success and practical feasibility of seed-based restoration efforts, while ensuring the efficient and sustainable use of seed resources sourced from donor meadows.

Improving germination outcomes may require targeted pre-treatments that enhance seed responsiveness when viability or dormancy cues have been altered by storage, handling, or environmental variability. Seed priming offers one such approach, helping seeds overcome physiological constraints associated with storage, handling, or suboptimal germination cues (Paparella et al., 2015; Akram et al., 2025; Takeda et al., 2025; Zhao et al., 2025). Hormo-priming with gibberellic acid (GA_3, the most widely used bioactive form of gibberellins), in particular, stimulates germination by shifting the hormonal balance between abscisic acid (ABA), which maintains dormancy, and GA, which promotes cell elongation and embryo growth (Finch-Savage and Leubner-Metzger, 2006). By activating these early metabolic and signaling pathways, GA_3 can trigger germination at this pivotal and sensitive life stage in a range of terrestrial species (Tsegay and Andargie, 2018; Khan et al., 2020; Ahmad et al., 2021; Perera et al., 2025). In contrast, the application of GA_3 to seagrasses has been only marginally investigated and limited to a few species, such as *Zostera noltii*, and *Posidonia australis* (Loques et al., 1990; Glasby et al., 2015). For *Z. marina*, a first attempt assessed GA_3 effects over a wide concentration range (10, 50, 500, and 1000 mg L^{-1}) and reported highly variable or inhibitory responses above 20 mg L^{-1} (Pieraccini et al., 2025c). However, the consistency and magnitude of GA_3 effects on *Z. marina* germination have not yet been systematically evaluated.

Here, we evaluated GA_3 priming as a means to promote *Z. marina* seed germination, with the broader aim of improving the reliability and efficiency of seed-based restoration. By enhancing germination success, GA_3 priming can help maximize the use of limited seed stocks and potentially reduce the number of seeds required to achieve target restoration densities. Building on previous work that tested light and GA_3 effects, we refined the dose–response curve by testing lower GA_3 concentrations under light-controlled conditions.

In this study, first, we tested ten GA_3 concentrations ranging between 0–20 mg L^{-1} on seeds from two reproductive seasons (2021 and 2022) of an intertidal annual population in Hamburger Hallig, Wadden Sea, Germany. Our aim was to identify optimal dose ranges and to assess intergenerational and treatment-specific variability. Second, we tested three selected GA_3 concentrations (3, 9, and 20 mg L^{-1}) on seeds from two subtidal, perennial populations in the Northwestern Atlantic – Buzzards Bay and Vineyard Sound, Cape Cod, Massachusetts – to evaluate the broader applicability of GA_3 priming across populations in different environmental settings. While our design does not isolate ecological or biogeographic effects, these distinct populations offer a useful test of GA_3 priming across contexts. Together, these experiments provide a broad assessment of GA_3's potential to enhance germination in *Z. marina*.

2 Materials and methods

2.1 Seed collection

Seeds of *Zostera marina* were collected from three meadows differing in habitat and life-history strategy: (1) an intertidal meadow in the Northeastern Atlantic, Wadden Sea, Hamburger Hallig, Germany (dominated by annual phenotypes) (Davies et al., 2026), and (2) two subtidal, perennial meadows in Buzzards Bay and Vineyard Sound, Cape Cod, Massachusetts, USA (Naushon Island and The Knob) (Figure 1). In the Wadden Sea, reproductive shoots were harvested during low tide at Hamburger Hallig (54°35′ 52.3″ N, 8°48′47.3″ E) in late August 2021 and 2022, coinciding with peak seed maturity. In Massachusetts, shoots were collected by snorkeling in late April–early June 2024, during peak seed development (Naushon Island: 41°31'04.9"N, 70°42'02.2"W; The Knob: 41°32'31.4"N, 70°39'27.4"W) (Figure 1).

Shoots were held in aerated tanks under near-field temperature (15 ± 1°C) and natural light conditions for 30 days to allow seed maturation and natural release. Mature seeds were identified by color (cyan to dark-brown), full development (intact seed coat), and negative buoyancy, which is assumed to correspond to completion of embryo development (Dooley et al., 2013; Xu et al., 2016, Xu et al., 2020). Although spathe maturation is asynchronous within a meadow, collections were timed before natural seed release, when most spathes contained fully developed seeds, and seed release occurred naturally under controlled conditions. Seeds were stored in autoclaved seawater — natural seawater at 30 PSU for Wadden Sea seeds and artificial seawater at 40 PSU (Instant Ocean) for Northwestern Atlantic seeds, the latter adjusted to a higher salinity improving storage, following recommendations from the Global Seagrass Nursery Network (workshop series 2022–2025) and recent literature (Xu et al., 2020; Thomson, 2023). Storage was conducted at 4 ± 1°C in darkness with gentle aeration to prevent stratification and anoxia. To limit *Phytophthora* proliferation, seeds were twice treated during storage with overnight rinses in 0.2 ppm $CuSO_4$ solution (Govers et al., 2017). Both natural and artificial seawater were refreshed regularly (every 2–3 weeks) to maintain water quality and oxygenation.

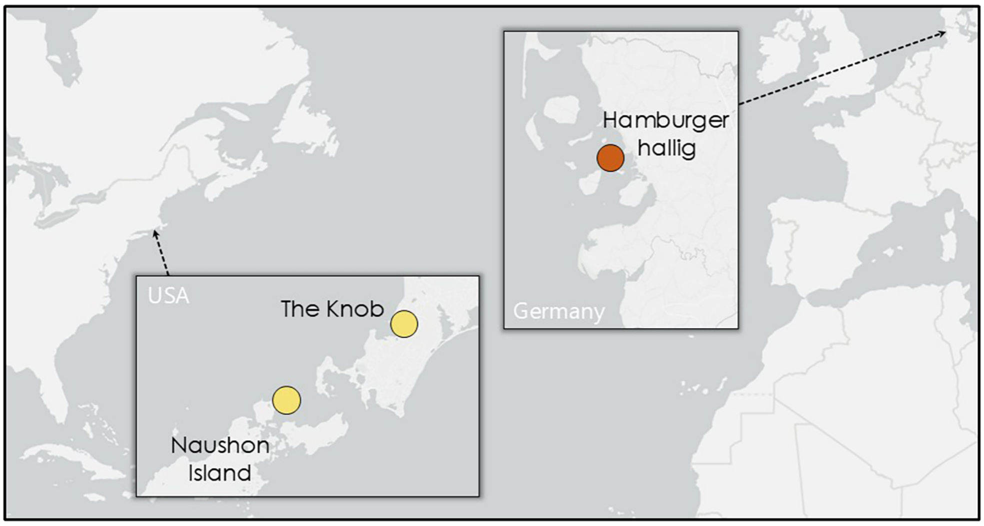

FIGURE 1
Geographic origin of *Zostera marina* seeds used in the GA_3 priming experiments. Northeastern Atlantic population: Hamburger Hallig, Wadden Sea, Germany (red dot) and Northwestern Atlantic populations: Naushon Island and The Knob, Massachusetts, USA (yellow dots). Insets show the regional context within Europe and North America.

2.2 Seed sterilization

Sterilization treatment was included to reduce microbial contamination (e.g. molds and bacteria) prior to experimental start, and to evaluate whether this commonly applied procedure influences germination success. Because only a subset of seed batches were sterilized, the design also allowed testing whether GA_3 priming could mitigate any potential negative effects of sterilization on seed viability. Sterilization was applied to seeds from the Northeastern Atlantic population (cohort S2022 and S2021) and the Northwestern Atlantic populations (Naushon Island and The Knob).

The sterilization protocol involved immersing seeds in 70% v/v ethanol (EtOH; Chem Lab NV) for 2 minutes, followed by treatment with 5% sodium hypochlorite (NaClO; Sigma-Aldrich) in sterilized seawater (SSW; 30 PSU) for 20 minutes. Seeds were then rinsed five times with SSW to remove residual chemicals. SSW was prepared by adjusting natural seawater salinity to 30 PSU with Milli-Q water and autoclaving at 121 °C, 15 psi, for 20 minutes.

2.3 Germination experiments

We conducted two complementary experiments to evaluate the effects of GA_3 priming on dormant *Zostera marina* seeds under controlled laboratory conditions. In both experiments, seeds were individually plated in 96-well plates, each well containing 100 µL of treatment solution (GA_3 in autoclaved seawater, hereafter SSW) or SSW alone for controls. The 96-well format was used consistently to prevent cross-contamination, but the number of seeds per plate differed between experiments. Plates were incubated at 10 ± 1°C with a 12:12 h light–dark photoperiod under full-spectrum LED lighting (100–120 µmol m^{-2} s^{-1}), simulating natural spring conditions for each region. All seeds had undergone a cold storage period (0–4°C) in darkness for 3 months, to mimic natural overwintering and primary dormancy release in the field.

2.3.1 Northeastern Atlantic population

This experiment, performed in spring 2023, aimed to identify optimal GA_3 concentrations and assess responses across seed cohorts of different ages and sterilization treatments. Seeds were collected from an intertidal, annual population at Hamburger Hallig, Germany (Figure 1), during two reproductive seasons: a 2022 cohort, tested within 6 months of collection, and a 2021 cohort (1-year-old seeds), stored for ~16 months before testing.

A total of 525 seeds were used: 175 from 2021 (all sterilized-S2021) and 350 from 2022 (half sterilized - S2022, half non-sterilized – NS2022). Ten GA_3 concentrations (0, 1, 3, 5, 7, 9, 11, 13, 15, 17, and 20 mg L^{-1}) were tested, with 15 replicates per concentration (distributed across plates). The experiment was repeated three times at two-week intervals to account for temporal variation, and data from all replicates were pooled for analysis.

The selected storage durations (six and sixteen months) corresponded to the natural seasonal cycle at the collection site, where seed release occurs around September and germination typically begins in March–April. Experiments started on March 15th and were repeated three times at two-week intervals to capture potential short-term temporal variation in germination responses under identical laboratory conditions, reflecting natural germination timing. This *Z. marina* population represents an annual ecotype. While the *Z. marina* seed bank is generally transient, seeds can remain viable beyond one year, albeit with declining viability (Yue et al., 2019; Pieraccini et al., 2025a). Testing both seed ages allowed assessment of whether GA_3 priming could enhance germination even in older, less viable seeds. Data from all replicates were pooled for analysis, as no significant variation among experimental runs was detected.

2.3.2 Northwestern Atlantic populations

This experiment tested whether GA_3 priming responses observed in the Northeastern Atlantic population were consistent in ecologically and geographically distinct *Z. marina* populations. Reproductive shoots were harvested from the field in July 2024 from two subtidal, perennial meadows in Buzzards Bay and Vineyard Sound (along Cape Cod, Massachusetts), at the locations of Naushon Island and The Knob (Figure 1). Reproductive shoots bearing seeds were maintained in ambient seawater tanks until seeds were naturally released. Seeds were then collected from the bottom of the tanks in October 2024.

Seed collection and handling followed the same procedures as the Northeastern Atlantic population experiment. Seeds were stored for approximately six months under controlled conditions (4 ± 1°C) to mimic natural overwintering before testing. This period corresponds to the typical duration between autumn seed release and spring germination at these latitudes. The experiment started on April 3, 2025.

For each site, seeds were randomly assigned to one of three GA_3 concentrations (3, 9, and 20 mg L^{-1}) or a control. For The Knob population, each treatment included 60 replicates, and for Naushon Island, each treatment included 30 replicates. Culture conditions matched those described above (section 2.3.1). While these populations differ from the Wadden Sea population in environmental setting, life history traits, and genetic background, the design does not allow these factors to be disentangled; instead, their inclusion provides a test of GA_3 priming across diverse contexts.

2.4 Monitoring

Both experiments with seed lots from Northeastern and Northwestern populations ran for 40 days, with germination observations conducted three times per week. Germination was defined as the emergence of the cotyledon from the seed coat (Liu et al., 2023; Pieraccini et al., 2025a).

2.5 Seed viability

At the conclusion of each experiment, the viability of non-germinated seeds was determined using a 2, 3, 5-Triphenyl-

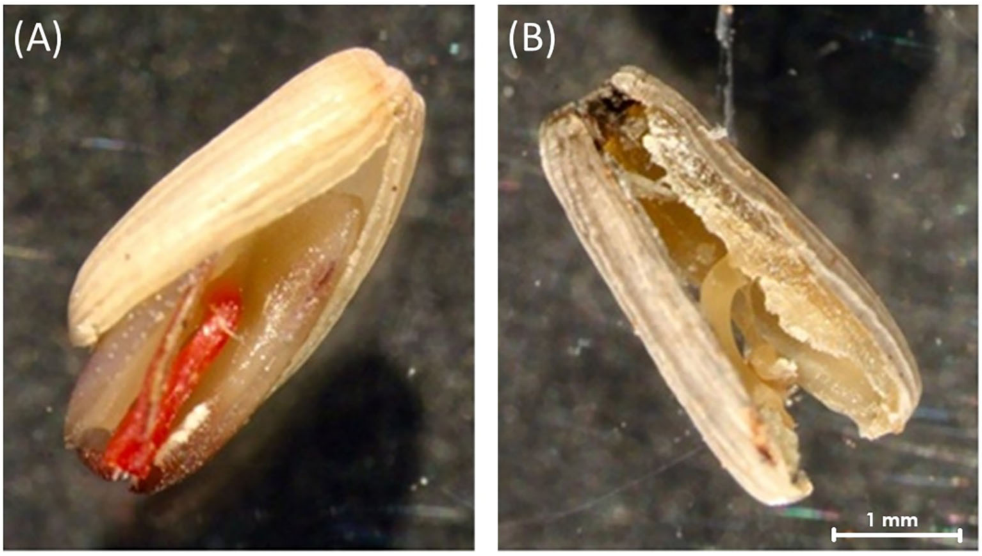

FIGURE 2
TTC seed staining. **(A)** TTC-stained and **(B)** unstained *Z. marina* seeds.

Tetrazoliumchloride (TTC) assay, following the methodology described by Lakon (1949). Seeds were punctured with a syringe needle and submerged in 100 µL of 1% TTC solution for 48 hours. Seed viability was determined based on the characteristic red staining of metabolically active tissues using TTC. Final germination percentages were calculated as the proportion of germinated seeds relative to the total number of viable seeds, as determined by TTC staining. This adjustment ensured that observed differences among treatments reflected germination performance rather than variation in seed viability (Figure 2). Seed viability results are presented in Supplementary Table S1. TTC assessment was conducted at the end of the 40-day experiment; seeds were not pre-selected by TTC at T_0.

2.6 Statistical analysis

To assess the effects of GA_3 treatments on final germination outcomes, penalized logistic regression models with Firth correction were fitted using R (v4.0.2; R Core Team, 2020). Analyses were performed separately for the Northeastern and Northwestern Atlantic experiments. In the Northeastern Atlantic experiment, explanatory variables included seed cohort, GA_3 concentration, and their interaction. In the Northwestern Atlantic experiment, the model included site, GA_3 concentration, and their interaction. Model selection was based on Akaike Information Criterion (AIC), with non-significant terms removed via stepwise backward elimination. *Post-hoc* comparisons between treatment groups were conducted using estimated marginal means with Tukey adjustment. Although total seed numbers differed slightly among populations and treatments, penalized logistic regression with Firth correction provides unbiased parameter estimates even with unequal or moderate sample sizes (Firth, 1993; Heinze and Puhr, 2010). This approach ensures robust inference despite unbalanced data, preventing small-sample bias and overestimation of effect sizes commonly observed in standard maximum-likelihood models.

Germination timing, the period until germination occurs, was analyzed using Cox proportional hazards models fitted with the survival and survminer packages (Therneau and Grambsch, 2000). Survival curves were constructed to visualize the germination process over time. The proportional hazards assumption was verified, and models were selected based on AIC. Hazard ratios (HRs) were used to estimate the relative likelihood of germination (germination likelihood) at any given time, that is, the probability that a seed will germinate at a specific time point given that it has not yet germinated. Pairwise differences between concentrations were assessed using adjusted estimated marginal means (Lenth, 2022).

All germination results are presented as mean germination (%) ± binomial confidence intervals % (Wilson method), based only on viable seeds as determined by TTC staining, unless stated otherwise. Seed viability assessments were used to adjust final germination percentages and ensure consistency in reporting across all experimental groups.

3 Results

3.1 Germination response to GA_3 concentrations (Northeastern Atlantic population)

Across all tested concentrations, seed germination (%) showed substantial variation, with clear differences observed between GA_3 concentrations and seed groups (Figure 3). The highest germination was recorded in the non-sterilized 2022 (NS2022) cohort, where

GA_3-treated seeds reached up to 64.3% germination at 13–20 mg L^{-1}, compared with 24% in NS2022 control (Figure 3). Several GA_3-treated groups within this cohort exhibited overall high mean germination (40.0%, 95% CI: 12.6–64.3%), with a marked increase above 9 mg L^{-1}. A Firth-corrected logistic regression statistically supported this trend ($\chi^2 = 48.3$, $p = 0.03$). In NS2022 cohort, the odds of germination increased sharply above 9 mg L^{-1}, reaching a 3.4- to 3.8-fold increase relative to controls ($p < 0.01$).

Both sterilized cohorts (S2021 and S2022) exhibited lower baseline germination (average of 10–15% in controls), however, seeds responded positively to GA_3 exposure. The strongest effects occurred at 3–5 mg L^{-1}, and 17–20 mg L^{-1} in S2021 cohort, and at 9 mg L^{-1}, and 20 mg L^{-1} in S2022, where germination probabilities rose by approximately 2- to 3-fold compared with control seeds ($p < 0.01$). This indicates that GA_3 treatment partially alleviated reduced viability or dormancy in sterilized (S2022) and older (S2021) seed cohorts.

On average, NS2022 seeds achieved significantly higher germination probabilities than both sterilized cohorts ($p < 0.01$). In contrast, mean germination in S2021 and S2022 cohorts generally remained below 40%, increasing only under GA_3 treatment. Control treatments showed much lower germination percentages: 14.6% (95% CI: 8.4–24.3%) in S2022 and 10.7% (95% CI: 5.5–19.7%) in S2021 (Figure 3), reinforcing the role of seed age and sterilization in limiting germination.

Germination timing, defined as the period until germination occurs, varied substantially across seed cohorts and GA_3 concentrations. GA_3-treated seeds germinated earlier and more rapidly than control seeds across seed cohorts, with the strongest effect observed in the NS2022 cohort. Similar trends were observed in S2021 and S2022, where GA_3 treatment led to earlier germination onset compared to control seeds.

A Cox proportional hazards model supported these trends, with an overall significant, positive effect of GA_3 treatment on germination likelihood (Hazard Ratio (HR) = 1.94, $p = 0.047$), this indicates that GA_3-treated seeds were nearly twice as likely to germinate at any given time compared to controls. Seed cohort also had a strong influence on germination likelihood, with both S2021 and S2022 germinating at lower hazard rates compared to NS2022, respectively HR = 0.48 ($p = 0.09$) and HR = 0.669 ($p = 0.30$), suggesting reduced germination timing. Despite these differences, GA_3 treatment supported reduced germination timing even in these seed cohorts. In both S2021 (older seeds) and sterilized S2022 seeds, germination timing improved under GA_3 exposure.

Concentration-specific effects were equally informative (Supplementary Table S2). In the NS2022 cohort, germination likelihood increased sharply above 9 mg L^{-1}, peaking at 20 mg L^{-1} where seeds were almost three times more likely to germinate compared with controls (HR = 2.91, $p < 0.001$). The 17 mg L^{-1} treatment also produced a strong acceleration (HR = 1.94, $p = 0.048$), while a weaker, non-significant positive trend was detected at 13 mg L^{-1} (HR = 1.86, $p = 0.063$). Lower concentrations (≤ 7 mg L^{-1}) had inconsistent or even slightly inhibitory effects in some cohorts, suggesting that low GA_3 concentrations may delay germination rather than stimulate it.

3.2 Germination response to GA_3 concentrations (Northwestern Atlantic populations)

Mean germination (%) in the Northwestern Atlantic populations was consistently higher than those observed in the Northeastern Atlantic population (Figure 4). Seeds from the Naushon Island population exhibited the highest germination in GA_3-treated seeds, reaching 88.0% (95% CI: 70.1–95.8) germination under 9 mg L^{-1} GA_3, and maintaining similarly high outcome under 20 mg L^{-1} (78.6%, 95% CI: 60.5–89.8%). Seeds from The Knob population showed slightly lower values overall, with a maximum mean germination of 69% (95% CI: 55.9–79.7%) at 20 mg L^{-1}. Seeds treated with 3 mg L^{-1} GA_3 showed similar success as controls in both sites.

A penalized logistic regression model revealed no statistically significant differences between concentrations or sites ($p > 0.28$).

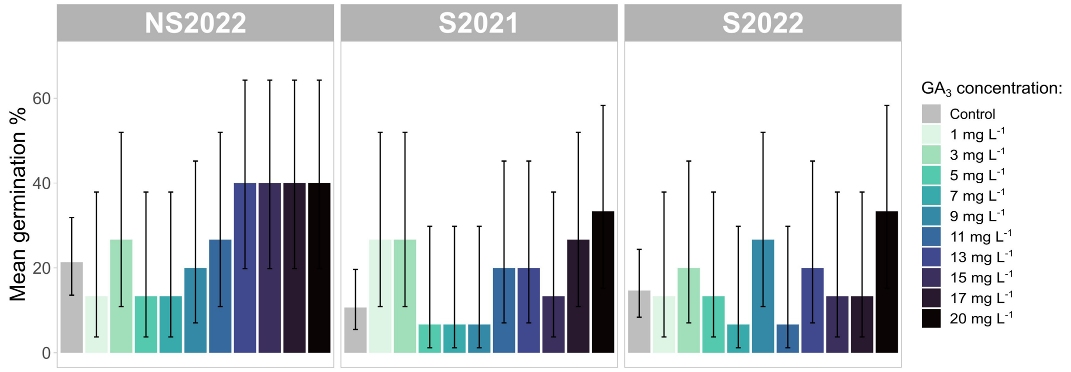

FIGURE 3
Mean seed germination (%) of *Z. marina* across ten GA_3 concentrations (1–20 mg L^{-1}), shown for three seed cohorts: NS2022, S2021, and S2022. Bars represent mean germination values, with binomial 95% confidence intervals shown as error bars. Colors range from light to dark blue to indicate increasing GA_3 concentration (1–20 mg L^{-1}), while the grey bar represents the control treatment (0 mg L^{-1}).

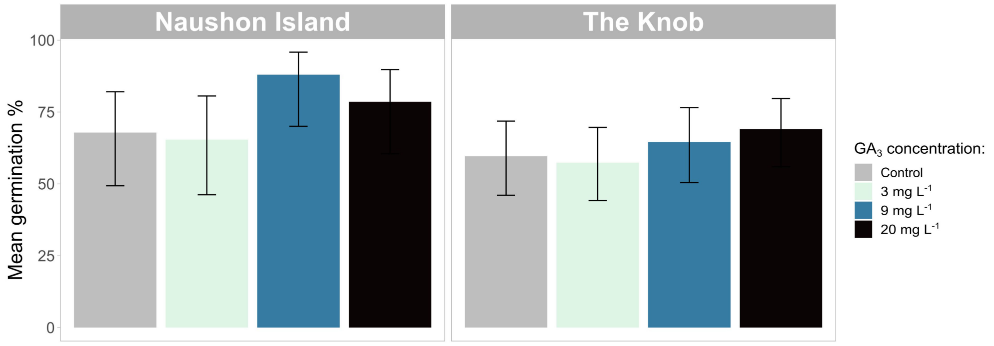

FIGURE 4
Mean seed germination (%) of *Z. marina* from two Northwestern Atlantic populations (Naushon Island and The Knob) across four GA_3 concentrations (3, 9, and 20 mg L^{-1}) and a control. Bars represent mean germination values, with binomial 95% confidence intervals shown as error bars.

However, model coefficients aligned with observed trends, with both 9 and 20 mg L^{-1} treatment positively affecting germination (respectively, estimate = 1.14 and estimate = 0.62), particularly in Naushon Island, whereas control and 3 mg L^{-1} treatments had slightly negative estimates.

Germination occurred more rapidly and synchronously across both Northwestern Atlantic populations, with most GA_3-treated seeds germinating within 10 to 15 days regardless of treatment (Figure 5). Cumulative germination curves showed near-complete germination by day 20 in all cases, with GA_3-treated seeds generally reaching maximal germination sooner than controls. This effect was particularly evident in the Naushon Island seeds, which showed reduced germination timing across all GA_3 concentrations compared to controls. A similar trend was observed in the The Knob site, though differences were less pronounced.

A Cox proportional hazards model supported these patterns, with a positive, concentration-dependent effect of GA_3 on germination likelihood. Seeds treated with 20 mg L^{-1} were 46% more likely to germinate earlier than controls (HR = 1.46, p = 0.051, Supplementary Table S3), while 9 and 3 mg L^{-1} also showed modest positive effects (HR = 1.36 and 1.31, respectively; $p > 0.05$). Despite high baseline viability in both populations, GA_3 exposure further reduced germination timing, particularly at higher concentrations, reinforcing its consistent effect even in population with high-viability seed batches.

4 Discussion

4.1 GA_3 priming enhanced germination success and reduced germination timing

This study investigated the potential of GA_3 priming to enhance seed germination in *Zostera marina* across seed cohorts from different population contexts (encompassing both geographic and ecological variation), as well as a range of post-harvest handling conditions. Building on earlier work testing the combined effects of GA_3 and light spectra on germination dynamics (Pieraccini et al., 2025c), we tested GA_3 priming on seeds from geographically and ecologically distinct populations. Seed cohorts were subjected to

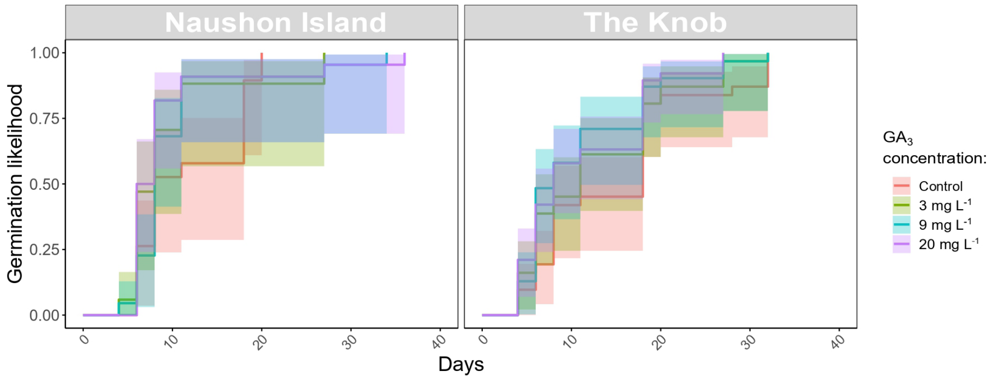

FIGURE 5
Model-predicted germination likelihood of *Z. marina* seeds from two Northwestern Atlantic populations (Naushon Island and The Knob) treated with gibberellic acid (GA_3). Curves represent the modeled probability of germination over time for each GA_3 concentration (Control, 3, 9, and 20 mg L^{-1}). Shaded areas indicate 95% confidence intervals. The term *germination likelihood* refers to the cumulative probability of germination events predicted from the fitted time-to-event model, corresponding to the progression of cumulative germination through time.

different storage duration and/or sterilization treatments to identify concentrations that consistently promote germination. In doing so, we aimed to assess not only the direct effects of GA_3, but also how variation in population context, storage conditions, and pre-treatment history influence germination outcomes, and whether GA_3 priming can mitigate factors that limit seagrass restoration success such as low germination rates, dormancy, and seed-quality decline that limit seagrass restoration success.

Our results demonstrate that GA_3 priming enhanced *Z. marina* germination across Northeastern and Northwestern Atlantic populations, improving both germination success and timing. Including populations from contrasting habitats and life histories provides a valuable test of GA_3 priming under varied pre-treatment histories. GA_3 priming consistently improved germination across concentrations, although no clear dose-dependent trend emerged. Similar non-linear responses have been reported in both terrestrial and seagrass systems (e.g. Glasby et al., 2015);, suggesting that germination is highly sensitive to specific, optimal GA_3 concentrations rather than following a linear relationship. Seed traits such as size or mass may influence germination responses (Dooley et al., 2013; Barak et al., 2018); however, in this study, all treatments were applied within the same seed cohort, minimizing trait-related variation among GA_3 concentrations. Priming treatments should therefore be optimized to achieve reliable germination while avoiding inhibitory effects, as observed in this study (Figures 3 and 4), and previously reported for species like *Z. marina*, *Zostera noltii* and *Posidonia australis* (Loques et al., 1990; Conacher et al., 1994; Pieraccini et al., 2025c). These results align with the known role of GA_3 in breaking dormancy through hormonal signaling (Rademacher, 2015) and confirm its potential to stimulate latent germination in *Z. marina*.

Mechanistically, GA_3 in terrestrial species promotes dormancy release by stimulating α-amylase activity and activating GA-regulated genes that facilitate endosperm weakening and embryo growth (Toyomasu et al., 1998). However, the molecular pathways underpinning GA_3 responses in seagrasses remain largely uncharacterized (Pieraccini et al., 2025a). The variability observed among seed cohorts and geographically distinct populations suggests underlying physiological or genomic differences (Olsen et al., 2004; Boström et al., 2014), potentially linked to environmental plasticity, local adaptation (Olsen et al., 2016), or seed characteristics such as size and mass. Determining whether these differences reflect ecotypic variation or storage-induced physiological changes, or other unmeasured factors will be important for refining priming strategies for seagrass restoration.

4.2 Seed origin and handling are critical

Seed responses to GA_3 varied strongly between seed cohort and seed populations. In *Z. marina* research and restoration, seed storage is commonly adopted, as seeds are often harvested before natural release and maintained under controlled, cold conditions (typically 0–4 °C) until use (Yue et al., 2019; Gamble et al., 2021), although storage duration and conditions remain unstandardized across studies and species. This cold stratification allows seeds to complete ripening and experience a required period of dormancy while being preserved for the following season. Nevertheless, prolonged storage has been associated with reduced germination (Dooley et al., 2013; Xu et al., 2020), likely due to the induction of secondary dormancy (Brady and McCourt, 2003; Finch-Savage and Leubner-Metzger, 2006). Prolonged cold, dark storage can induce secondary dormancy through shifts in the ABA/GA hormonal balance, suppression of cell wall-loosening enzymes, or changes in seed-associated microbial communities; GA_3 may counteract these constraints by re-activating endosperm-weakening and embryo growth pathways (Brady and McCourt, 2003; Hourston et al., 2022). Consistently, control seeds from the S2021 cohort (seed stored for approximately 16 months) showed lower germination than those from the more recent S2022 cohort, likely reflecting a decline in seed viability during extended storage, consistent with the transient nature of the *Z. marina* seed bank (Dooley et al., 2013; Jarvis et al., 2014; Xu et al., 2020). Despite this, both cohorts responded positively to GA_3 priming, with increased germination observed across most tested concentrations. Similar outcomes have been reported in terrestrial species (Muhamad, 2013; Tsegay and Andargie, 2018) but have remained largely unexplored in seagrasses.

In addition to storage, we examined the impact of surface sterilization using seeds collected in the same year (S2022 vs NS2022). Sterilization is commonly used to prevent microbial growth during storage, yet our results indicate that mild sterilization can negatively affect germination (Marion and Orth, 2008; Xu et al., 2019; Yue et al., 2019). These findings reinforce concerns that subtle stressors, such as sterilization, cold storage, or handling, may compromise seed viability or induce secondary dormancy (Cumming et al., 2017; Unsworth et al., 2023). However, while seed storage treatments, including those targeting pathogens or algae, are often necessary, they may inadvertently compromise germinability (Xu et al., 2019; Yuki et al., 2024). This observation aligns with recent discussions in *Z. marina* research emphasizing the risks of over-sterilization (Pieraccini et al., 2025c). Even minor microbial shifts during storage can impair seed emergence, as observed in the seagrass *Posidonia australis* (Conacher et al., 1994).

Interestingly, GA_3 priming mitigated the negative effects of sterilization, improving germination in sterilized seeds and enhancing germination success in non-sterilized seeds. These results suggest that hormo-priming can partially rescue germination capacity, under storage conditions, that may reduce seed viability, and mild sterilization stress.

4.3 GA_3 priming across distinct populations

To assess the reproducibility of GA_3 priming across ecologically and genetically distinct populations, we tested seeds from an intertidal, annual Wadden Sea population and two subtidal, perennial, populations from the Northwestern Atlantic. These populations differ in habitat type (intertidal annual vs. subtidal perennial) and environmental regimes and have been shown to be genetically distinct (Olsen et al., 2004). Although a mechanistic investigation of the specific genes regulating germination was beyond the scope of this study, population-level divergence may involve differences in hormonal signaling, dormancy regulation, or stress-response mechanisms. Such genetic and physiological adaptations likely reflect adaptation to temperature, salinity, hydrodynamic, and light regimes, which shape seed dormancy depth and germination variability (Hughes et al., 2009; Kaldy et al., 2015; Cumming et al., 2017). However, the molecular basis of germination regulation in marine angiosperms, and in *Z. marina* in particular, remains largely unexplored.

Despite ecological and genetic differences, GA_3 priming generally enhanced germination across populations, with improvements in either final germination or germination timing observed for most concentrations. This suggests a degree of physiological convergence in germination response, even though the relative contributions of ecological and biogeographic factors cannot be fully disentangled in this study. Interestingly, the intertidal annual population, which depends entirely on seed recruitment for meadow renewal (Van Katwijk et al., 2016; van Katwijk and van Tussenbroek, 2023), exhibited lower baseline germination compared to the subtidal perennial populations. This likely reflects an adaptive strategy to cope with the highly dynamic intertidal zone, where deeper dormancy prevents premature germination during short favorable periods (Jarvis et al., 2014). This potential dormancy depth strategy may support the maintenance of persistent seed banks, buffering population renewal across years with poor recruitment (Marion and Orth, 2009; Jarvis et al., 2014; Sgambelluri et al., 2025).

In contrast, the higher baseline germination rates observed in the Northwestern Atlantic populations may reflect several interacting factors. Their larger seed size and greater nutrient reserves (Baskin and Baskin, 2000; Jørgensen et al., 2019) likely support germination and establishment under less hydrodynamic and more stable subtidal conditions. These perennial populations also rely more on clonal propagation for persistence, which reduces selective pressure to maintain prolonged dormancy or extensive seed banks (Jarvis and Moore, 2010; Kendrick et al., 2017). Additionally, the transition from storage at 40 PSU to 30 PSU may have acted as a salinity cue, as a decline in salinity can stimulate germination in *Z. marina* (Xu et al., 2016). Consequently, part of the higher baseline germination observed in the northwestern populations could reflect this salinity-driven response rather than inherent population-level differences.

Interestingly, even within this region, germination success varied between sites: seeds from Naushon Island achieved the highest germination under both control and GA_3 treatments, whereas seeds from The Knob exhibited lower overall germination but still responded positively to priming. These differences may reflect local environmental conditions or genetic differences influencing dormancy depth and hormonal sensitivity (Finch-Savage and Leubner-Metzger, 2006; Pieraccini et al., 2025c).

Despite the already high viability of Naushon Island seeds, GA_3 priming further improved germination dynamics, particularly by accelerating germination timing. Primed seeds germinated with increasing synchrony, showing up to a 1.4-fold reduction in germination time at mid- and high GA_3 concentrations. This pattern is consistent with findings from terrestrial systems, where priming increases germination synchrony and narrows emergence windows (Cañizares et al., 2025).

While earlier and more synchronized germination can accelerate establishment, it is not universally beneficial. In natural systems, simultaneous germination may increase exposure to storms (Valdemarsen et al., 2010), tropical cyclones (Baker et al., 2024), marine heatwaves (Pieraccini et al., 2025b), or predation (Carroll et al., 2019). Under certain ecological contexts, however, synchrony can be advantageous: mass germination may reduce predation losses through predator satiation (Bonal et al., 2007), promote canopy growth that reduces seabed hydrodynamics (Lefebvre et al., 2010), stabilize sediment for new recruits, and allow seedlings to pre-empt competitors such as algae or invertebrates. Conversely, asynchronous germination spreads risk across time, representing a classic form of ecological bet-hedging in unpredictable environments.

In restoration contexts, however, synchrony can become desirable. Coordinated germination enhances seedling uniformity, simplifies nursery management, and allows practitioners to align emergence with optimal temperature and light windows to avoid potential heat stress (e.g. heatwaves), cyclones or storms. Thus, while the adaptive value of synchrony depends on environmental and site contexts, controlled germination timing through GA_3 priming can offer a practical advantage for restoration under increasing climate variability (Duarte et al., 2018; Unsworth et al., 2023).

Overall, these findings reinforce that GA_3 can enhance germination success, speed and synchrony, traits that, when harnessed under managed conditions, could improve the predictability and scalability of seed-based seagrass restoration.

4.4 Ecological relevance and restoration implications

Seed-based seagrass restoration efforts continue to face persistent bottlenecks at the germination and seedling stages (Unsworth et al., 2023; Van Katwijk et al., 2016). While effective conservation remains the first and most essential strategy to safeguard existing meadows, restoration can complement these efforts when losses cannot be reversed through protection alone and where environmental conditions are actively managed to provide suitable habitat within an ecosystem-based management framework. In recent years, *Zostera marina* seed-based restoration has gained momentum, supported by advances in ecological and biological understanding (Valdez et al., 2020; Rehlmeyer et al., 2024; Cronau et al., 2025; Preston et al., 2025) and by improvements in seeding techniques, including large-scale broadcast seeding demonstrated in the Virginia Coastal Bays (e.g. Orth et al., 2006, Orth et al., 2012, Orth et al., 2020). Further technological developments—such as Dispenser Injection Systems (DIS) (Govers et al., 2022; Gräfnings et al., 2023b), hessian seed bags

(Unsworth et al., 2019; Rautenbach et al., 2024), autonomous seeding robots (Unsworth and Rees, 2025), and large-scale seed-ball burial in northern China (Xu et al., 2023), are enhancing efficiency, spatial precision, and scalability of seed deployment across diverse restoration settings (Unsworth et al., 2019; Gräfnings et al., 2023a, Gräfnings et al., 2023b). The physiological improvements demonstrated here through GA_3 priming could complement these technological advances by enhancing seed readiness and germination reliability prior to large-scale deployment. Regional- and global-scale studies provide important context for restoration, offering insights across different species, regions, and environmental conditions (Gräfnings et al., 2024; Coals et al., 2025b). At the same time, technological developments – such as seeding robots engineered for intertidal and subtidal planting (Unsworth and Rees, 2025; Robocean, n.d.; Ulysses, n.d.) – along with growing involvement of non-governmental organizations and social enterprises are enhancing restoration scalability. These initiatives integrate local knowledge, e.g. from fisherman, while creating new job opportunities in coastal communities.

As global interest in seagrass restoration increases, attention is increasingly directed to each developmental stage of the plant. Different seed triggers, such as temperature shifts, salinity pulses, or freshwater rinses, have been explored to mimic seasonal, field-like conditions. However, results have often been mixed (Brenchley and Probert, 1998; Alexandre et al., 2006; Fernández-Torquemada and Sánchez-Lizaso, 2013; Kaldy et al., 2015). Mechanical scarification and acid rinsing aim to replicate natural seed coat abrasion, while microbial priming is an emerging approach to stimulate natural seed signaling pathways (Harrison, 1991; Cumming et al., 2017). More recently, new hormonal treatments are also being tested to better understand dormancy regulation and plant responses (Pieraccini et al., 2025c, Pieraccini et al., 2025a).

Within this context, our study focused on one pivotal stage, germination, to support the growing momentum toward reproducible and efficient seed-based restoration practices. GA_3 priming significantly improved both germination success and timing under controlled conditions. Insights from the Global Seagrass Nursery Network (Workshop series 2021–2025) indicate that seed processing workflows vary widely across restoration programs, research groups, and organizational facilities, reflecting differences in local logistics, infrastructure, and restoration objectives. Typically, reproductive shoots are collected from donor meadows and seeds are separated, cleaned, and then stored under temperature- and salinity-controlled conditions until sowing or experimental use. Prior to planting, seeds are sorted and prepared for deployment using methods such as DIS, hessian bags, seed ball, or seed broadcasting. This diversity in approaches underscores the need for simple, transferable techniques that integrate into existing frameworks without extensive modification. GA_3 priming can be incorporated as an additional pre-treatment within existing seed-handling workflows. This involves short-term immersion (e.g. 24 h) of stored seeds in GA_3 solutions (typically 9–20 mg L^{-1}) prior to sowing, using temperature-controlled seawater tanks or similar incubation facilities already common in seed storage laboratories. At the time of writing, 500 mg of GA_3 (≥ 90% purity, Sigma-Aldrich) costs approximately €82, corresponding to €1.48 per liter of a 9 mg L^{-1} solution and €3.28 per liter of a 20 mg L^{-1} solution. Given that a *Zostera marina* seed weighs about 2 mg, and assuming gentle agitation or aeration to ensure uniform exposure, approximately 25, 000 seeds could be primed per liter of solution. This makes GA_3 priming a low-cost and easily transferable procedure that can be implemented within restoration nurseries using standard laboratory equipment and trained personnel. Similar to recent spatial deployment strategies that enhance seeding density and patch survival (Infantes and Moksnes, 2018; Cronau et al., 2025), hormo-priming offers a potential upstream intervention to increase seed readiness and synchrony before field planting.

However, the variability observed across populations and seed cohorts suggests that a one-size-fits-all approach may be suboptimal. Factors such as seed origin, age, and pre-treatment history influence germination responses and should be considered when developing protocols. While this variation may pose a challenge, our results indicate that certain concentrations, particularly 9 – 20 mg L^{-1}, consistently enhanced germination across different contexts, suggesting they could serve as a robust starting point for field testing and restoration trials. Ultimately, integrating GA_3 priming into nursery workflows has clear potential to reduce losses from poor germination, increase propagule availability for outplanting, and improve the consistency and resilience of seedling production. Based on our results, GA_3 treatment can enhance germination up to twofold compared to untreated controls. If similar outcomes can be achieved under field conditions, this improvement could reduce the number of seeds needed to reach target meadow coverage, thereby lowering collection and deployment costs and minimizing impacts on donor meadows. Increased seed-use efficiency would also support the long-term sustainability of seed-based restoration programs. In conclusion, GA_3 priming represents a conceptually simple and cost-effective pre-treatment, offering a practical upstream intervention to enhance seed readiness and support seagrass restoration.

Data availability statement

The raw data supporting the conclusions of this article are made available in the Supplementary Materials.

Author contributions

RP: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Project administration, Validation, Visualization, Writing – original draft, Writing – review & editing. GD: Investigation, Methodology, Writing – review & editing. TD: Methodology, Resources, Writing – review & editing. NK: Methodology, Resources, Supervision, Validation, Writing – review & editing. SM: Investigation, Methodology, Validation, Writing – review & editing. LP: Investigation, Methodology, Validation, Writing – review & editing. FP: Investigation, Methodology, Writing – review & editing. MT: Funding acquisition, Resources, Supervision, Writing – review & editing. TV: Data curation, Resources, Supervision, Validation, Visualization, Writing – review & editing. AV: Conceptualization, Funding acquisition, Methodology, Project administration, Resources, Supervision, Writing – review & editing.

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Conflict of interest

The authors declared that this work was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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