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Seed Germination and Seedling Development of *Rudbeckia hirta* L. Under Different Light Conditions, Surface-Sterilization Treatments, and Growing Substrates

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Abstract

Black-eyed Susan (*Rudbeckia hirta* L.) is a North American native species used in ornamental horticulture, pollinator landscapes, and ecological restoration. This study evaluated the effects of light, surface sterilization, and substrate on germination behavior and early seedling establishment. Germination was evaluated for 20 days under two contrasting light treatments: continuous light and continuous darkness. Five surface-sterilization protocols were compared for microbial contamination, followed by seedling establishment in Pro Mix BX and Sunshine Mix #4 for 60 days under controlled conditions. Final germination percentage did not differ significantly between continuous light (89.6%) and continuous darkness (68.0%). Mean germination time was significantly lower under continuous darkness (4 days) than under continuous light (9 days), while germination speed was significantly higher under darkness (26.8%) than under light (11.6%) ($p < 0.05$). Surface sterilization reduced contamination relative to the nonsterilized control, which exhibited an approximately 100% contamination rate. Treatments with 60% commercial bleach for 10 min (T1), 10% commercial bleach with Tween 20 for 20 min (T2), and 30% commercial bleach for 5 min followed by 0.2% Plant Preservative Mixture™ (T4) produced the lowest contamination levels. Principal component analysis showed that PC1 and PC2 explained 52.3% and 25.2% of the variance, respectively, accounting for 77.5% of the cumulative variance. T4 × Sunshine Mix #4 was associated with the highest plant height and leaf number, whereas T2 × Sunshine Mix #4 showed higher leaf chlorophyll content. These results provide information for seed propagation and early establishment of *R. hirta*.

Keywords: aseptic establishment; Asteraceae; black-eyed Susan; growing substrate; ornamental horticulture; seedling development

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1. Introduction

The Asteraceae family, comprising more than 1600 genera and 25,000 species worldwide, represents one of the largest and most diverse families of flowering plants, occurring across a broad range of ecosystems and accounting for nearly 10% of all flowering plant species [1]. Species within this family are widely recognized for their diverse floral characteristics, medicinal applications, and morphological diversity [2]. Members of the Asteraceae also exhibit considerable variation in growth habit, ranging from herbaceous species to shrubs and trees [3]. Native species such as blanket flower (*Gaillardia pulchella*) and lanceleaf coreopsis (*Coreopsis lanceolata*) provide pollen and nectar resources for flower-visiting insects and other beneficial pollinators [4]. Many Asteraceae occur as herbaceous forbs that

contribute to habitat biodiversity and provide floral resources throughout multiple growing seasons [4–6]. These plant–pollinator interactions support the inclusion of Asteraceae species in ecological restoration, where the recovery of pollination services can contribute to the reestablishment of ecosystem functions following a disturbance [7]. In addition to their ecological importance, the Asteraceae includes economically important food crops, particularly vegetables within the genus *Lactuca*, such as head, leaf, and romaine lettuce, which generated approximately USD 5.84 billion in 2025, while artichoke production was valued at approximately USD 86.6 million [8,9].

Among the ornamental taxa within the Asteraceae, the genus *Rudbeckia* comprises more than 20 species of herbaceous flowering plants native primarily to North America, including annual, biennial, and perennial species [10]. Members of the genus vary in growth habit, habitat, and longevity and are characterized by conspicuous composite inflorescences that contribute to their high aesthetic value and widespread use in the floriculture industry, which constitutes a significant component of global horticulture [11,12]. In 2024, *Rudbeckia* ranked 14th among herbaceous perennial crops in the U.S. floriculture industry, with sales valued at approximately USD 11.7 million, increasing from USD 10.2 million in 2019 [13,14]. *Rudbeckia hirta* L., commonly known as black-eyed Susan, is a North American native wildflower characterized by polychromatic inflorescences with conical, convex, or flattened centers and can reach approximately 1 m in height [15,16]. The species is characterized by its adaptability to relatively dry conditions and an extended flowering period from mid-summer through fall, traits that contribute to its use in ornamental and low-input landscapes [17]. In addition to its ornamental value, *R. hirta* is frequently included in conservation and restoration plantings, where it has demonstrated successful establishment and consistent flowering [18]. Successful establishment of native forbs can increase floral richness and resource availability, which has been positively associated with the abundance of native bees and honey bees in restored habitats [19].

Despite its broad distribution and adaptability, the establishment and development of *R. hirta* are influenced by environmental conditions across different stages of its life cycle. Across its geographic range, the species occurs under diverse climatic and edaphic conditions that can affect seedling establishment, vegetative growth, and reproductive development [20,21]. Seed germination may be influenced by dormancy-related mechanisms and environmental cues, including temperature and water availability [22]. Light availability is also an important environmental factor that can influence both the extent and temporal dynamics of seed germination [23]. A previous study has shown that germinability is higher under light conditions, whereas germination occurs more rapidly under continuous darkness for *R. hirta*, *R. triloba*, *E. perfoliatum*, and *L. spicata* [24]. Dormancy and germination responses in *R. hirta* can also vary among seed lots. Recent evaluations of commercial seed lots reported differences in germination and dormancy status, while temperature and dormancy-breaking treatments such as GA_3 also modified germination responses [25]. Similar variation has been reported within Asteraceae, with light promoting the release of seed dormancy in *Crassocephalum crepidioides* in association with differences in abscisic acid levels [26]. In *Scolymus hispanicus*, germination increased in response to light and cold storage [27], while studies with *Solidago* species showed that the effect of cold stratification on germination can vary among taxa [28]. These studies indicate that light sensitivity and dormancy responses are species-specific and not uniform within Asteraceae. Under certain conditions, germination percentages of approximately 60% have been reported, while differences among seed lots and responses to water stress further demonstrate variability in the germination performance of *R. hirta* [25]. Variations in temperature and seasonal conditions can modify flowering phenology and flowering duration [29], while light availability and spectral quality can regulate flowering time and reproductive development in *R. hirta* [30].

In addition to these environmental factors, the growth and development of *R. hirta* are influenced by nutrient availability, with an appropriate supply of macro- and micronutrients contributing to vegetative performance and plant establishment [31]. Nutrient management is particularly relevant in ornamental production because excessive fertilization can increase nutrient losses without proportional improvements in plant performance. Commercial growing substrates may differ in physical properties and incorporated amendments that can influence plant growth and performance. Silicon-containing amendments have been widely associated with increased plant width and stem diameter in ornamental species, including *R. hirta* and other flowering plants [32]. Similarly, substrates inoculated with biologically active amendments such as arbuscular mycorrhizal fungi (AMF) may influence vegetative development. AMF colonization has been associated with changes in morphological traits, such as plant height, leaf area, and aboveground biomass in members of the Asteraceae family [33]. Studies with container-grown ornamentals have demonstrated that optical measurements of leaf chlorophyll and canopy greenness can provide rapid, non-destructive indicators of plant nutrient status and support more precise fertilization management [34–36].

Because *R. hirta* responds to environmental conditions across multiple developmental stages, reliable propagation requires a better understanding of the factors regulating seed germination and early seedling establishment. Although previous studies have examined dormancy, temperature, water availability, and light responses, information remains limited on how contrasting light conditions affect final germination and germination timing in *R. hirta* across subsequent stages of aseptic establishment and early seedling development. Despite its horticultural and ecological importance, information on suitable propagation conditions and their effects on the morphological and physiological development of *R. hirta* remains limited. Standardized propagation procedures are also limited, particularly for seed germination, aseptic establishment, and early seedling development. Seed-based propagation can yield variable responses because germination is governed by interactions between intrinsic seed characteristics and environmental factors, including dormancy, temperature, and light [37]. In ornamental horticulture, in vitro propagation has particular value because it enables the production of large quantities of uniform plant material while providing controlled culture conditions and facilitating season-independent production [38,39]. In vitro systems can also facilitate the establishment of plant material and enable evaluation of species-specific responses to environmental conditions and culture treatments [40,41]. However, successful aseptic establishment requires effective surface-disinfection procedures that suppress microbial contamination without adversely affecting seed viability, germination, or subsequent seedling development [42].

Given the limited information available on the propagation requirements of *R. hirta*, this study aimed to examine key factors affecting seed germination, aseptic establishment, and early seedling development under controlled conditions. The specific objectives were to (i) determine the effects of light conditions on seed germination and germination dynamics, (ii) evaluate seed surface-sterilization treatments for their effectiveness in reducing microbial contamination during in vitro establishment, and (iii) assess the morphological and physiological responses of seedlings derived from different sterilization treatments following establishment in two commercial peat-based substrates. We hypothesized that continuous light would increase final germination percentage but reduce germination speed. We further hypothesized that surface sterilization would reduce microbial contamination relative to the nonsterilized control and that seedling growth and leaf greenness would vary among sterilization treatment × substrate combinations. By examining these successive stages of propagation, the study provides insights for improving seed-based production of *R. hirta* and its establishment under controlled conditions, with potential applications in ornamental horticulture, native plant production, and ecological restoration.

2. Materials and Methods

Three sequential experiments were conducted to evaluate factors affecting seed propagation and early establishment of *Rudbeckia hirta*. Experiment I examined the effects of light conditions on seed germination. Experiment II evaluated seed surface-sterilization protocols for reducing microbial contamination during in vitro establishment. Experiment III assessed early seedling establishment and vegetative development in two commercial peat-based substrates using seedlings obtained following the sterilization treatments.

2.1. Plant Material

Seeds of 'Black-eyed Susan' (*Rudbeckia hirta* L.) were obtained from Eden Brothers (Arden, NC, USA). Seeds were maintained in their original packaging under dry conditions at 21 °C until use. Prior to each experiment, seeds were visually inspected, and damaged or malformed seeds were excluded.

2.2. General Experimental Conditions

All aseptic procedures were performed in a laminar flow hood (Purair LF Series HLF72, Air Science USA, Fort Myers, FL, USA) to minimize airborne microbial contamination during seed sterilization, handling, and in vitro establishment. All three experiments were conducted in a controlled environment growth chamber (GEN1000 GE, Conviron, Winnipeg, MB, Canada) maintained at 27 °C, consistent with temperatures previously reported for in vitro culture of *R. hirta* [43]. Photosynthetically active radiation (PAR; µmol m^{-2} s^{-1}) and relative humidity (RH; %) were monitored throughout the experimental period using a HOBO MX2308 Temp/RH/PAR Data Logger (Onset Computer Corporation, Bourne, MA, USA). Across the experimental period, daily mean PAR was 59.51 ± 0.09 µmol m^{-2} s^{-1}, while daily mean RH was 46.89 ± 0.08%. Daily mean PAR and RH values recorded during the three experiments are presented in Supplementary Figure S1.

2.3. Experiment I: Effect of Light on Seed Germination

The effect of light on seed germination was evaluated under two conditions: continuous light and continuous darkness. These contrasting treatments were selected to isolate the effect of light availability on germination under controlled conditions rather than to simulate a natural day–night cycle. Seeds used in this experiment were not subjected to surface sterilization before placement in the Petri dishes. Seeds were placed in sterile 90 mm Petri dishes lined with filter paper moistened with sterile distilled water. Each Petri dish contained 25 seeds and represented one experimental unit. The experiment followed a completely randomized design with four independent replicates per light treatment, for a total of 100 seeds per treatment, consistent with replication schemes used in previous controlled seed germination studies [44–46].

Petri dishes assigned to the continuous light treatment were maintained under a 24 h photoperiod provided by cool white fluorescent lamps. For the continuous darkness treatment, Petri dishes were completely wrapped in two layers of aluminum foil immediately after sowing to prevent light exposure. Germination was monitored daily for 20 days for both treatment groups. Dishes maintained under continuous darkness were evaluated under dim green safe light within a laminar flow hood to minimize light exposure that could influence seed photoreceptor activity. The aluminum foil was removed only for the time required to record germinated seeds, after which the dishes were immediately rewrapped. A seed was considered germinated when the radicle extended at least 2 mm beyond the seed coat. Germination percentage (GRP, %), mean germination time (MGT, days), germination speed (GSP, %), and germination synchrony (SYN) were calculated using the GerminaR 2.1.6 package [46].

2.4. Experiment II: Optimization of Seed Surface Sterilization

Unlike Experiment I, in which non-surface-sterilized seeds were germinated on sterile filter paper moistened with sterile distilled water, Experiment II evaluated aseptic establishment on MS medium. Nutrient media used for in vitro culture can support microbial growth when microorganisms are present on or within plant material, making surface disinfection important for aseptic establishment [47,48]. Surface disinfectant concentration and exposure time can affect seed germination and early seedling development [49,50]. Five surface-sterilization protocols were evaluated for their effectiveness in reducing microbial contamination while maintaining the germination capacity of *R. hirta* seeds during in vitro establishment (Table 1). Sterile distilled water without chemical disinfection (T0) served as the control. Treatment T1 consisted of 60% commercial bleach (8.25% sodium hypochlorite) for 10 min following Ozel et al. [51]. Treatment T2 consisted of 10% commercial bleach supplemented with Tween 20 (one drop per 100 mL) for 20 min according to Sugii [52]. Treatment T3 consisted of 3% hydrogen peroxide (H_2O_2) for 2 h following Barampuram et al. [53]. Treatment T4 consisted of 30% commercial bleach for 5 min followed by 0.2% Plant Preservative Mixture™ (PPM) based on Ozel et al. [51].

Table 1. Surface-sterilization protocols evaluated for *Rudbeckia hirta* seeds during in vitro establishment. Treatments differed in disinfectant type, concentration, exposure time, and the use of antimicrobial or surfactant additives.

Treatments	Surface Disinfection	Reference Basis
T0	No sterilization, only distilled water rinse	—
T1	60% commercial bleach for 10 min	Ozel et al. [51]
T2	10% bleach solution + Tween 20 for 20 min	Sugii [52]
T3	3% hydrogen peroxide (H_2O_2) for 2 h	Barampuram et al. [53]
T4	30% bleach solution for 5 min + 0.2% PPM	Ozel et al. [51]

Following surface sterilization, seeds were rinsed three times with sterile distilled water under aseptic conditions and inoculated onto hormone-free Murashige and Skoog (MS) basal medium. Cultures were maintained under a 16 h photoperiod, consistent with previous in vitro culture conditions reported for *R. hirta* [43]. Each Petri dish contained 25 seeds and represented one experimental unit. The experiment was conducted using a completely randomized design with four replicates per sterilization treatment, resulting in 20 experimental units and 500 seeds in total. Seed germination and microbial contamination were recorded daily for 20 days.

Sterilization efficiency was assessed using germination percentage (GRP, %) and contamination rate (CR, %). The CR metric was calculated following Munoz-Salas et al. [54], based on an adaptation of the germination speed approach described by Lozano-Isla et al. [46]. Visible fungal or bacterial growth on the culture medium was recorded as contamination. The index was calculated as

$$CR(\%) = \left(\frac{\sum_{i=1}^{k} C_i}{\sum_{i=1}^{k} C_i D_i} \right) \times 100$$

where C_i is the number of seeds that became contaminated on day i, D_i is the corresponding number of days after culture initiation, and k represents the final observation day. Higher CR values indicated earlier and faster contamination, whereas lower values reflected delayed contamination and improved sterilization efficiency during the 20-day evaluation period.

2.5. Experiment III: Seedling Establishment in Commercial Substrates

Seedlings obtained following the sterilization treatments were transplanted into two commercial peat-based growing substrates to evaluate early establishment and vegetative development. The substrates were Pro Mix BX (Premier Tech Horticulture, Rivière-du-Loup, QC, Canada), composed of 75–85% peat moss, perlite, calcitic and dolomitic limestone, wetting agent, and mycorrhizae, and Sunshine Mix #4 (Sun Gro Horticulture, Agawam, MA, USA), composed of Canadian sphagnum peat moss, perlite, dolomitic lime, wetting agent, and the RESiLIENCE® silicon amendment.

The experiment was conducted using a completely randomized design with a 4 × 2 factorial arrangement comprising four sterilization treatments (T1–T4) and two growing substrates (Pro Mix BX and Sunshine Mix #4). The nonsterilized control (T0) was excluded from this experiment because all seedlings from this treatment developed microbial contamination during in vitro establishment. For each treatment (e.g., sterilization treatment × substrate combination), five seedlings were evaluated, resulting in a total of 40 experimental units.

Individual seedlings were transplanted into square plastic containers (10 × 10 × 11 cm) filled with the corresponding substrate and maintained under a 16 h photoperiod [43]. Plants were irrigated as needed throughout the 60-day experimental period without supplemental fertilization.

At 60 days after transplanting, plant height (cm) and the number of fully expanded leaves were recorded. Leaf greenness was assessed using a SPAD 502 Plus chlorophyll meter (Konica Minolta, Osaka, Japan) and an atLEAF + chlorophyll meter (FT Green LLC., Wilmington, DE, USA). Measurements were collected from the third fully expanded leaf, with three readings taken per plant and averaged for statistical analysis, following the protocol described by Lozano-Isla et al. [55].

2.6. Statistical Analysis

Statistical analyses were performed in R version 4.6.0 [56] within a reproducible Quarto workflow (Supplementary Material S1). Prior to analysis, datasets were screened for potential outliers and evaluated for normality and homogeneity of variance. Outlier detection was conducted with the outliers_remove () function from the inti 0.7.2 package [57].

Experiment I and Experiment II each included four independent replicates per treatment, whereas Experiments III included five independent replicates per treatment combination, respectively. The number of replicates was selected with reference to Piepho et al. [58], balancing statistical precision with logistical feasibility.

For Experiments I and II, treatment effects were analyzed using linear mixed-effects models according to the following general model:

$$Y_{ij} = \mu + T_i + R_j + \varepsilon_{ij}$$

where Y_{ij} represents the response variable, μ is the overall mean, T_i is the fixed effect of treatment, R_j is the random effect of replicate, and ε_{ij} is the residual error. In Experiment I, light condition was included as the fixed effect and replicate as a random effect. In Experiment II, surface-sterilization treatment was included as the fixed effect and replicate as a random effect.

For Experiment III, surface-sterilization treatment, substrate, and their interaction were included as fixed effects according to the following factorial model:

$$Y_{ijk} = \mu + S_i + M_j + (SM)_{ij} + R_k + \varepsilon_{ijk}$$

where Y_{ijk} represents the response variable, μ is the overall mean, S_i is the effect of surface-sterilization treatment, M_j is the effect of substrate, $(SM)_{ij}$ is the interaction between surface-sterilization treatment and substrate, R_k is the random effect of replicate, and ε_{ijk} is the residual error.

Linear mixed effects models were fitted with the lme4 2.0-6 package [59]. Fixed effects were assessed by Type III analysis of variance, with degrees of freedom and *p*-values estimated using the Kenward–Roger approximation implemented in the car 3.1-5 package [60]. When significant treatment effects were detected, estimated marginal means were compared using Tukey-adjusted pairwise comparisons at $\alpha = 0.05$ with the emmeans 2.0.4 package [61].

Principal component analysis (PCA) was conducted using the FactoMineR 2.16 R package [62] to evaluate associations among morphological and physiological variables and differences among treatments. Treatment similarity was further evaluated by hierarchical cluster analysis based on Euclidean distances and Ward's minimum variance method. Model diagnostic plots, PCA ordinations, and hierarchical clustering dendrograms were generated with the ggplot2 4.0.3 package [63] and additional packages from the tidyverse collection 2.0.0 package [64].

3. Results

3.1. Effect of Light on Seed Germination

To evaluate the response of *R. hirta* seeds to light conditions, germination was monitored daily for 20 days under continuous light and continuous darkness (Figure 1). Final germination percentage (GRP) was numerically higher under continuous light (89.6%) than under continuous darkness (68.0%); however, no significant difference was detected between treatments. Mean germination time (MGT) differed significantly between light conditions ($p < 0.05$), with seeds maintained in continuous darkness exhibiting a lower MGT (4 days) than those exposed to continuous light (9 days). Germination speed (GSP) was also significantly affected by light conditions ($p < 0.05$), reaching 26.82% under continuous darkness compared with 11.58% under continuous light. Germination synchrony (SYN) was 0.20 under continuous darkness and 0.26 under continuous light, with no significant difference between treatments.

3.2. Effect of Surface Sterilization on Microbial Contamination

Surface sterilization significantly affected contamination rate (CR) during the 20-day culture period (Figure 2a). The non-sterilized control (T0) exhibited the highest CR (100%) and was significantly higher than all sterilization treatments ($p < 0.05$). Among the disinfection protocols, T3 exhibited an intermediate CR of 31.67%, significantly exceeding the values observed for T1, T2, and T4 ($p < 0.05$). In contrast, T1 (3.25%) and T2 (7.67%) showed the lowest CR values and did not differ significantly from one another ($p > 0.05$), while no contamination was recorded in T4 (0.00%). Representative cultures at the end of the 20-day evaluation period visually reflected these differences, with extensive microbial growth in T0, varying levels of contamination in T1–T3, and no visible contamination in T4, where germinated seedlings were observed (Figure 2b).

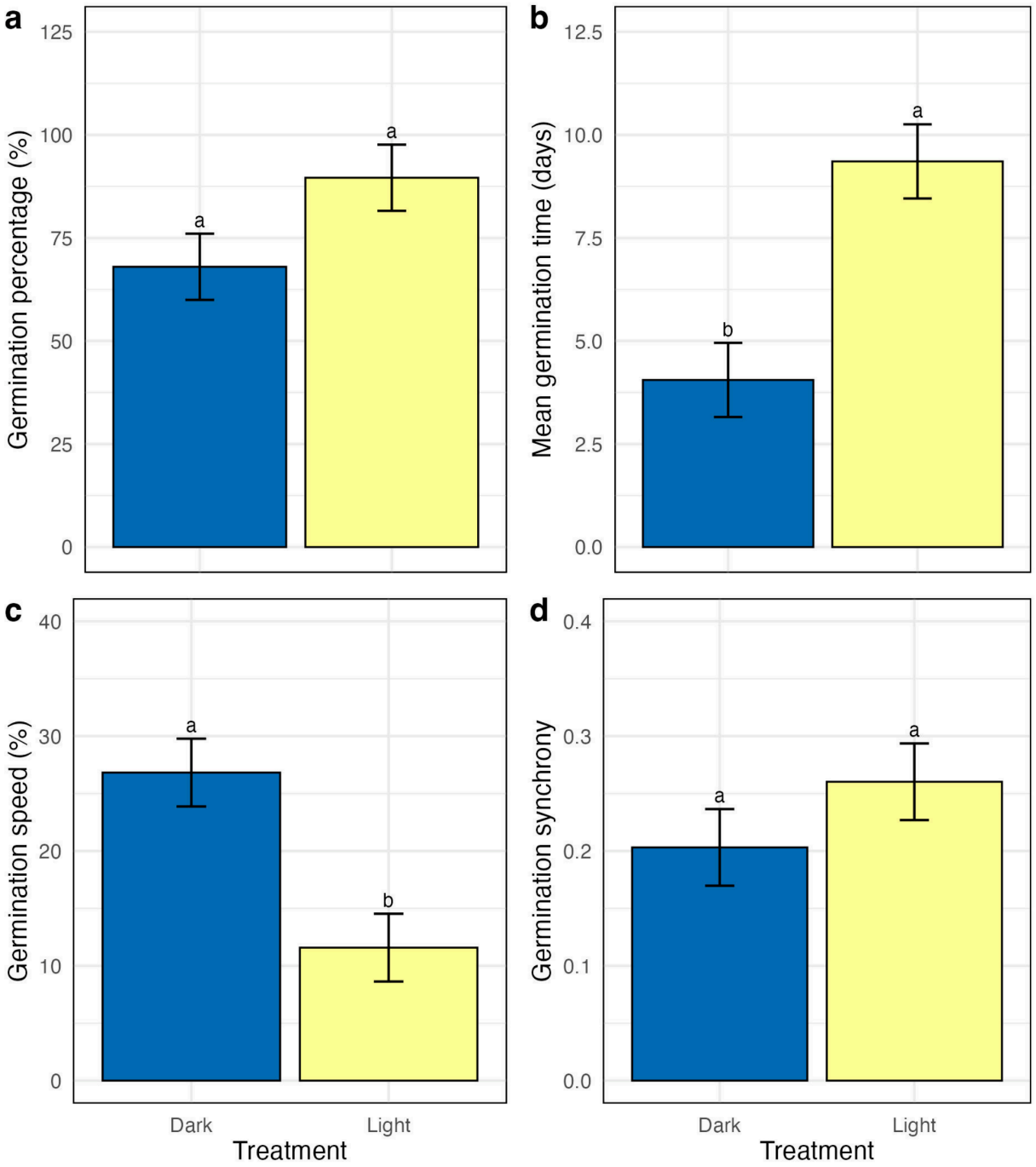


Figure 1. Effect of light conditions on seed germination of *Rudbeckia hirta*. Seeds were maintained under continuous darkness (Dark) or continuous light (Light) for 20 days. (**a**) Final germination percentage (GRP; %), (**b**) mean germination time (MGT; days), (**c**) germination speed (GSP; %), and (**d**) germination synchrony (SYN). Bars represent estimated marginal means ± SE. Different lowercase letters above bars indicate significant differences between light treatments ($p < 0.05$); bars sharing the same letter are not significantly different ($p > 0.05$).

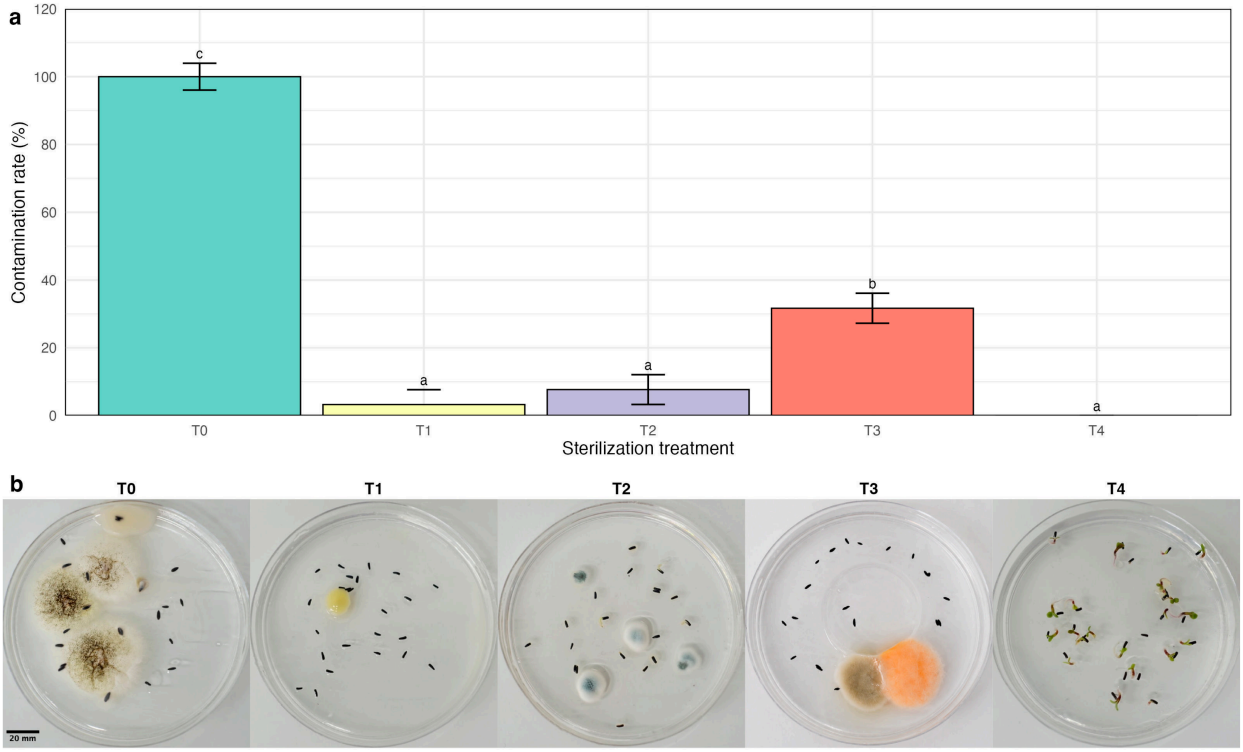


Figure 2. Effect of surface-sterilization treatments on microbial contamination of *Rudbeckia hirta* seeds during in vitro establishment: (**a**) Contamination rate (CR) of seeds subjected to five surface-sterilization treatments (T0–T4) during the 20-day evaluation period. (**b**) Representative cultures showing microbial contamination and seed germination after 20 days of in vitro culture for each sterilization treatment. T0, sterile distilled water rinse; T1, 60% commercial bleach for 10 min; T2, 10% commercial bleach supplemented with Tween 20 for 20 min; T3, 3% H_2O_2 for 2 h; and T4, 30% commercial bleach for 5 min followed by 0.2% Plant Preservative Mixture™. Statistical differences between light treatments were assessed using pairwise comparisons of estimated marginal means with Tukey's adjustment. Different lowercase letters indicate significant differences between treatments ($p < 0.05$).

3.3. Seedling Establishment and Vegetative Development

Seedling responses differed among sterilization treatment and substrate combinations (Table 2). Plant height ranged from 10.16 ± 6.57 cm in T3 × Sunshine Mix #4 to 16.18 ± 2.97 cm in T4 × Pro-Mix BX, with no significant differences among combinations ($p > 0.05$). SPAD and atLEAF values also did not differ significantly among combinations ($p > 0.05$). In contrast, leaf number differed among treatments. T4 × Sunshine Mix #4 produced 7.20 ± 1.92 leaves, while T1 × Sunshine Mix #4 and T4 × Pro-Mix BX produced 3.20 ± 1.92 and 3.80 ± 0.84 leaves, respectively. The remaining combinations showed intermediate values (Table 2).

The first two principal components explained 77.5% of the total variation, with PC1 accounting for 52.3% and PC2 for 25.2% (Figure 3). Plant height and leaf number showed similar loading patterns, while SPAD and atLEAF were also closely associated (Figure 3a). T2 × Sunshine Mix #4 was associated with SPAD and atLEAF, whereas T4 × Sunshine Mix #4 was positioned in the direction of plant height and leaf number (Figure 3b). These results indicate that the treatment combination associated with leaf greenness differed from that associated with morphological development.

Table 2. Morphological and physiological traits of *Rudbeckia hirta* seedlings after 60 days of establishment under different surface-sterilization treatment and growing substrate combinations. Values represent mean ± SD ($n = 5$). Different lowercase letters within each column indicate significant differences among treatment combinations according to Tukey pairwise comparisons ($p < 0.05$).

Sterilization	Substrate	Plant Height (cm)	Leaf Number	SPAD	atLEAF
T1	Pro-Mix BX	14.80 ± 4.55 a	5.60 ± 1.14 ab	29.27 ± 5.25 a	32.87 ± 5.03 a
T1	Sunshine Mix #4	10.70 ± 6.43 a	3.20 ± 1.92 a	30.03 ± 16.95 a	33.05 ± 19.18 a
T2	Pro-Mix BX	15.58 ± 1.34 a	5.40 ± 1.14 ab	30.57 ± 7.77 a	36.68 ± 8.78 a
T2	Sunshine Mix #4	13.80 ± 1.52 a	5.80 ± 0.84 ab	41.39 ± 5.57 a	40.60 ± 4.22 a
T3	Pro-Mix BX	12.26 ± 1.38 a	5.40 ± 0.55 ab	33.33 ± 9.12 a	32.99 ± 4.01 a
T3	Sunshine Mix #4	10.16 ± 6.57 a	4.40 ± 2.70 ab	28.94 ± 16.45 a	29.99 ± 17.25 a
T4	Pro-Mix BX	16.18 ± 2.97 a	3.80 ± 0.84 a	30.74 ± 1.53 a	33.85 ± 1.11 a
T4	Sunshine Mix #4	13.60 ± 4.06 a	7.20 ± 1.92 b	31.09 ± 5.75 a	31.03 ± 5.08 a

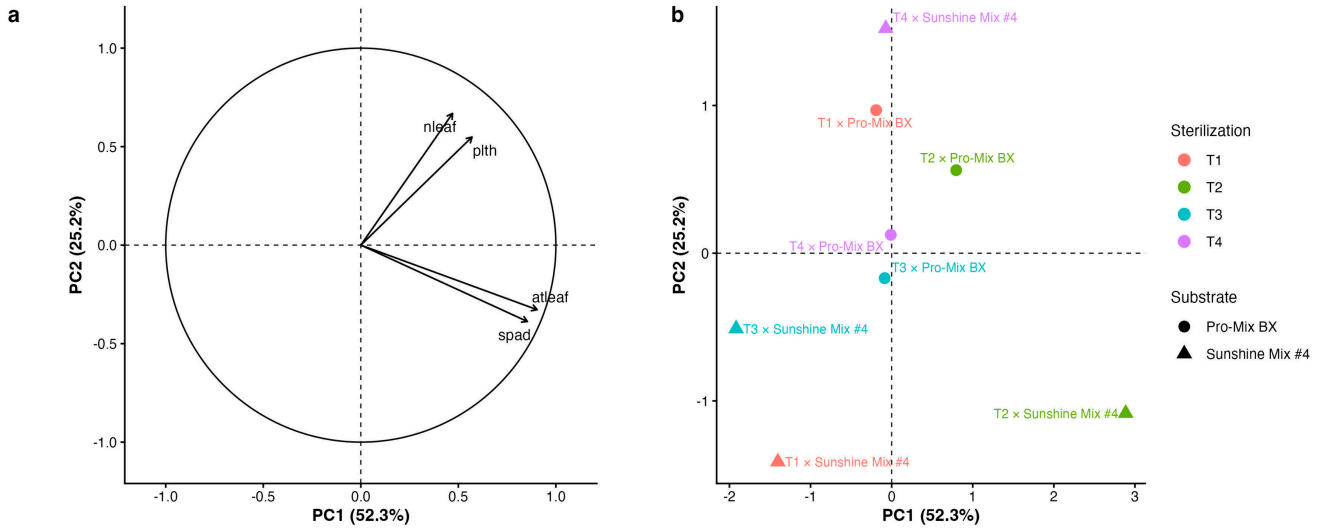


Figure 3. Principal component analysis (PCA) of morphological and physiological traits of *Rudbeckia hirta* seedlings after 60 days of establishment in a controlled environment growth chamber under different sterilization treatment and substrate combinations: (**a**) PCA of variables showing the relationships among plant height (plth), number of leaves (nleaf), and leaf greenness indices measured using SPAD and atLEAF. (**b**) PCA of individuals grouped by sterilization treatment (T1–T4) and growing substrate (Pro-Mix BX and Sunshine Mix #4). Colors represent sterilization treatments, while symbols indicate substrates (circles, Pro-Mix BX; triangles, Sunshine Mix #4) ($n = 40$).

4. Discussion

Rudbeckia hirta responded differently across the three propagation stages evaluated. Light affected germination kinetics, with continuous darkness reducing mean germination time and increasing germination speed without significantly changing final germination percentage, indicating that light mainly influenced germination timing. NaOCl-based sterilization treatments reduced microbial contamination during in vitro establishment, consistent with their use as surface disinfectants. Seedling morphology and leaf greenness varied among sterilization treatment and substrate combinations, showing that early establishment was influenced by both pre-establishment treatment and growing substrate. These results provide practical information for seed propagation, aseptic establishment, and early seedling development of *R. hirta*.

4.1. Effects of Light Conditions on Seed Germination

Light availability affected germination dynamics of *R. hirta* but did not significantly change final germination percentage. Seeds under continuous light reached 89.6% germination compared with 68.0% under continuous darkness (Figure 1). The value observed under light is consistent with Ermiş et al. [25], who reported germination of 89% after 4 days and 95% after 14 days in *R. hirta*. Germination responses in this species can also vary with temperature and stratification. Hoffman [65] reported 4% germination at 24 °C without stratification, while stratified seeds exposed to light reached 24–36% germination.

Continuous darkness reduced mean germination time from 9 to 4 days and increased germination speed from 11.58% to 26.82%. Ferreira et al. [66] reported mean germination times ranging from approximately 2.4 to more than 15 days among native Asteraceae, depending on species and temperature. Based on this classification, *R. hirta* shifted from an intermediate germination response under continuous light to a faster response under darkness. These results indicate that light affected germination timing more than final germination percentage under the conditions evaluated.

Light responses during germination vary among Asteraceae. Experiments with 9 grassland Asteraceae species showed that germination probability and speed varied among taxa in response to environmental conditions, emphasizing that germination kinetics can differ even among closely related species [67]. Similar variation has been reported among four annual Asteraceae from southwestern Australia, where three species with comparatively larger seeds germinated better in darkness, whereas germination of the smallest-seeded species was largely restricted to light [68]. In tallgrass prairie Asteraceae, *Rudbeckia laciniata* was classified as non-photoblastic because germinability changed little across light levels, whereas other species in the same family showed increased germination with increasing light availability [69]. Positive photoblasticity has also been reported in *Crepidiastrum denticulatum*, where germination was higher in light than darkness [70]. These interspecific differences indicate that a uniform light requirement cannot be assumed even among closely related taxa. For *R. hirta*, the present results suggest that light was not essential for final germination under the conditions tested, yet its absence substantially accelerated the germination process.

4.2. Effects of Surface Sterilization on Aseptic Seed Germination

Surface sterilization influenced the success of aseptic establishment in *R. hirta*. Although all sterilization treatments reduced contamination relative to the untreated control, the lowest contamination levels were observed with T1, T2, and T4, which remained below 8%, with no contamination recorded in T4 (Figure 2). In contrast, seeds treated with 3% H_2O_2 alone showed a higher contamination level of approximately 32%. These results indicate that NaOCl-based treatments provided more effective microbial control than H_2O_2 alone during in vitro establishment of *R. hirta* seeds.

The effectiveness of NaOCl observed in this study is consistent with its widespread use for aseptic establishment, although efficacy is dependent on concentration and exposure duration [71–73]. Within Asteraceae, *Cirsum pitcheri* seeds were surface disinfected with 0.25% sodium hypochlorite before controlled germination assays [74]. The absence of visible contamination in T4 agrees with findings by Bošnjak Mihovilović et al. [75], who reported reduced microbial contamination in wheat seeds treated with NaOCl and PPM without a significant effect on germination. Higher PPM concentrations, however, can negatively affect plant development [75,76]. In the present study, T1, T2, and T4 provided the lowest contamination levels, while H_2O_2 alone was less effective.

4.3. Effects of Sterilization Treatment and Substrate on Early Seedling Development

Early seedling development varied according to the combination of sterilization treatment and growing substrate (Table 2). Vegetative growth and leaf greenness did not respond similarly across the conditions evaluated, as combinations associated with increased plant height and leaf production differed from those associated with higher SPAD and atLEAF values (Figure 3). This response was reflected in the PCA, where plant height and leaf number were grouped in one direction, while SPAD and atLEAF were grouped in another along PC2. This response indicates that conditions favoring vegetative growth were not necessarily those favoring leaf greenness during the early establishment of *R. hirta*.

Growing medium-dependent differences in morphological and physiological traits have been reported in other horticultural species. In *Erythropalum scandens*, substrate composition differentially affected above-ground growth, root development, and chlorophyll accumulation, with peat-rich mixtures favoring shoot development while a perlite-containing substrate was associated with enhanced root development and chlorophyll content [77]. Similarly, peat-based substrate modifications affected plant height, leaf production, chlorophyll content, and photosynthetic performance in *Rhododendron delavayi* [78]. Within Asteraceae, Di Lonardo et al. [79] reported that the growth response of *Leucanthemum vulgare* depended on growing-medium composition: most of the evaluated peat-free mixtures supported plant growth and quality, while some mixtures improved biomass and biometric traits relative to the conventional peat-based medium.

In ornamental production, substrate composition similarly influenced germination, seedling uniformity, and plant quality in marigold and zinnia, highlighting the importance of the growing medium's physical and biological properties [80]. More recent work with native container plants has also shown that responses to substrate composition are species-specific rather than universally associated with one medium [81]. These findings are consistent with the broad separation observed among Sunshine Mix #4 combinations in the present PCA and the tighter grouping of several Pro-Mix BX combinations near the origin.

Within the present study, T2 in Sunshine Mix #4 was most strongly associated with SPAD and atLEAF, whereas T4 in Sunshine Mix #4 was positioned in the direction of plant height and leaf number. This divergence suggests that the combination favoring leaf greenness was not identical to that associated with more pronounced vegetative development. Similar dissociation among plant traits has been reported in substrate studies where chlorophyll accumulation, shoot growth, and root development responded to different proportions of peat, perlite, and other components [77]. Because both Pro-Mix BX and Sunshine Mix #4 are peat-based formulations but differ in amendments and physical characteristics, their contrasting positions in the PCA likely reflect differences in the root-zone environment experienced during early establishment. Responses to silicon amendments can also vary among species and measured traits. Costa et al. [82] reported species-specific responses to silicon application in landscape plants, with effects differing among plant height, leaf production, SPAD, atLEAF, and NDVI measurements. This interpretation is consistent with broader substrate research showing that water-holding capacity, aeration, nutrient retention, and substrate composition jointly influence seedling morphology and physiological performance [83].

The close positioning of SPAD and atLEAF in the PCA indicates that both instruments described a similar leaf greenness response across the evaluated combinations. Both meters estimate relative chlorophyll status using light transmittance at similar wavelengths [84,85]. Similar relationships between SPAD and atLEAF have also been reported in *Chrysanthemum* and *Lactuca sativa* [86,87]. The usefulness of non-destructive optical sensing has also been demonstrated within Asteraceae. Dunn et al. [88] reported that SPAD and NDVI measurements could be used to monitor nitrogen status in potted *Gaillardia aristata*, although the

strength of their relationships with leaf nitrogen varied with sampling time. These findings support the use of both instruments for non-destructive assessment of leaf greenness in *R. hirta*. The lower cost reported for atLEAF relative to SPAD may also make it a practical alternative when repeated measurements or larger numbers of plants are required [87].

Beyond the responses observed during germination and seedling establishment, optimization of propagation practices for *R. hirta* has broader relevance for ornamental and landscape production. Species of *Rudbeckia* combine ornamental value with adaptability to environmental conditions, characteristics that support their use in private and public landscapes and other managed green spaces [15]. In this context, reliable protocols or germination, aseptic establishment, and early seedling development can support more consistent production of plant material for ornamental horticulture, native plant production, and ecological restoration.

4.4. Limitations and Future Research

Despite the practical relevance of these findings, some limitations should be considered. *Rudbeckia hirta* is a North American native wildflower widely used in landscaping and recognized for its hardiness and adaptability to different growing conditions [89]. The present study focused on the effect of light availability on germination, although the response of *R. hirta* seeds may also depend on interactions with other environmental and seed-related factors. Future studies examining light together with temperature and dormancy status would provide a broader understanding of the factors regulating germination in this species.

Second, the sterilization experiment evaluated visible microbial contamination during a 20-day culture period but did not identify the microorganisms present. Future studies should identify fungal and bacterial contaminants and determine whether the protocols differ in their effectiveness against specific microbial groups. The absence of visible contamination should also be considered together with subsequent seedling performance, because antimicrobial treatments that effectively suppress contamination can adversely affect plant development at higher concentrations.

Finally, the seedling establishment experiment was limited to two commercial peat-based substrates and a 60-day period under controlled conditions. The PCA identified differences among treatment and substrate combinations, but the physical and chemical properties of the substrates were not independently evaluated. Future studies should include larger sample sizes, substrate characterization, root architecture, biomass accumulation, nutrient status, and longer-term survival and flowering. Evaluation of peat-reduced and peat-free substrates would also be relevant, particularly because alternative growing media have successfully supported growth of other perennial Asteraceae used for ornamental and restoration purposes.

5. Conclusions

This study identified stage-specific responses during seed propagation and early establishment of *Rudbeckia hirta*. Continuous darkness reduced mean germination time and increased germination speed relative to continuous light, whereas final germination percentage did not differ significantly between light conditions. Surface sterilization reduced microbial contamination, with the NaOCl-based T1, T2, and T4 protocols providing the lowest contamination levels. During subsequent establishment, leaf number differed among sterilization treatment × substrate combinations, whereas plant height, SPAD, and atLEAF showed no significant differences. PCA further identified distinct associations between morphological traits and leaf-greenness indices. These findings provide practical information for controlled seed propagation, aseptic establishment, and early seedling production of *R. hirta* for ornamental horticulture, native plant production, and ecological restoration.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/seeds5060071/s1, Figure S1: Daily mean photosynthetically active radiation (PAR; µmol m^{-2} s^{-1}) and relative humidity (RH; %) recorded during the experimental period from August to December 2025; Supplementary Material S1: Quarto-based statistical analysis workflow in R, including data processing, statistical analyses, and associated code and outputs.

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References

1. Rolnik, A.; Olas, B. The Plants of the Asteraceae Family as Agents in the Protection of Human Health. *Int. J. Mol. Sci.* **2021**, *22*, 3009. [CrossRef] [PubMed]
2. Nikolić, M.; Stevović, S. Family Asteraceae as a Sustainable Planning Tool in Phytoremediation and Its Relevance in Urban Areas. *Urban For. Urban Green.* **2015**, *14*, 782–789. [CrossRef]
3. Umber, F.; Zafar, M.; Ullah, R.; Bari, A.; Khan, M.Y.; Ahmad, M.; Sultana, S. Implication of Light and Scanning Electron Microscopy for Pollen Morphology of Selected Taxa of Family Asteraceae and Brassicaceae. *Microsc. Res. Tech.* **2022**, *85*, 373–384. [CrossRef] [PubMed]
4. Kalaman, H.; Wilson, S.B.; Mallinger, R.E.; Knox, G.W.; Kim, T.; Begcy, K.; Van Santen, E. Evaluation of Native and Nonnative Ornamentals as Pollinator Plants in Florida: II. Floral Resource Value. *HortScience* **2022**, *57*, 137–143. [CrossRef]
5. Deever, D.T.; Soley, N.M.; Fullin, K.; Wilsey, B.J. Recruitment Limitation of Early- and Late-flowering Grassland Forbs Can Be Overcome with Transplanting in Prairie Restorations. *J. Appl. Ecol.* **2024**, *61*, 223–235. [CrossRef]
6. Grman, E.; Zirbel, C.R.; Bauer, J.T.; Groves, A.M.; Bassett, T.; Brudvig, L.A. Super-abundant C4 Grasses Are a Mixed Blessing in Restored Prairies. *Restor. Ecol.* **2021**, *29*, e13281. [CrossRef]
7. Lybbert, A.H.; Cusser, S.J.; Hung, K.J.; Goodell, K. Ten-year Trends Reveal Declining Quality of Seeded Pollinator Habitat on Reclaimed Mines Regardless of Seed Mix Diversity. *Ecol. Appl.* **2022**, *32*, e02467. [CrossRef] [PubMed]
8. Agbede, I.K.; Philips Akinsulire, O.; Faboyo, Y.E.; Johnson Akinloye, A. Economically Important Species of Asteraceae: An Overview of Leaf Epidermal Micro-Morphology with Respect to Water Availability. *Acta Biol. Slov.* **2023**, *66*, 13–24. [CrossRef]
9. U.S. Department of Agriculture, National Agricultural Statistics Service. *Vegetables 2025 Summary*; USDA-NASS: Washington, DC, USA, 2026. Available online: https://www.nass.usda.gov/Publications/Todays_Reports/reports/vegean26.pdf (accessed on 20 July 2026).
10. Hind, N.; Lambkin, D. 1082. *Rudbeckia hirta* L. *Curtiss Bot. Mag.* **2023**, *40*, 487–502. [CrossRef]
11. Khoddamzadeh, A.A.; Munoz-Salas, M.N.; Cabrera, A.; Nageswara-Rao, M. Light–Fertilizer Interactions Regulate Morphophysiological Performance and Nutrient Dynamics in *Plumeria rubra* under Subtropical Nursery Conditions. *Front. Hortic.* **2026**, *5*, 1881853. [CrossRef]
12. Vardeman, E.; Lyles, J.T.; Quave, C.L. The Genus Rudbeckia: A Critical Review of Its Traditional Medicinal Uses, Phytochemistry, and Pharmacology. *J. Herb. Med.* **2022**, *31*, 100530. [CrossRef]

13. U.S. Department of Agriculture, National Agricultural Statistics Service. *2019 Census of Horticultural Specialties*; Special Studies, Part 3, Report AC-17-SS-3; U.S. Department of Agriculture: Washington, DC, USA, 2020; Volume 3. Available online: https://www.nass.usda.gov/Publications/AgCensus/2017/Online_Resources/Census_of_Horticulture_Specialties/HORTIC.pdf (accessed on 27 July 2026).
14. U.S. Department of Agriculture, National Agricultural Statistics Service. *2024 Census of Horticultural Specialties*; Special Studies, Part 3, Report AC-22-SS-3; U.S. Department of Agriculture: Washington, DC, USA, 2026; Volume 3. Available online: https://www.nass.usda.gov/Publications/AgCensus/2022/Online_Resources/Census_of_Horticulture_Specialties/HORTIC.pdf (accessed on 27 July 2026).
15. Horotán, K.; Orlóci, L.; Farkas, D.; Neményi, A.; Kisvarga, S. Exciting Black-Eyes Susan (*Rudbeckia* spp.): Breeding Challenges and Opportunities. In *Breeding of Ornamental Crops: Annuals and Cut Flowers*; Al-Khayri, J.M., Jain, S.M., Wani, M.A., Eds.; Advances in Plant Breeding Strategies; Springer Nature: Cham, Switzerland, 2025; Volume 6, pp. 221–261, ISBN 978-3-031-78652-5.
16. Jourdan, P. Genetic Resources of Herbaceous Ornamentals in North America. In *North American Crop Wild Relatives*; Greene, S.L., Williams, K.A., Khoury, C.K., Kantar, M.B., Marek, L.F., Eds.; Springer International Publishing: Cham, Switzerland, 2019; Volume 2, pp. 607–643, ISBN 978-3-319-97120-9.
17. Mircea, D.M.; Calone, R.; Shakya, R.; Saavedra, M.F.; Sestras, R.E.; Boscaiu, M.; Sestras, A.F.; Vicente, O. Use of Multivariate Analysis in Screening for Drought Tolerance in Ornamental Asteraceae Species. *Agronomy* **2023**, *13*, 687. [CrossRef]
18. Simanonok, S.C.; Otto, C.R.V.; Iovanna, R. Forbs Included in Conservation Seed Mixes Exhibit Variable Blooming Detection Rates and Cost-effectiveness: Implications for Pollinator Habitat Design. *Restor. Ecol.* **2022**, *30*, e13657. [CrossRef]
19. Bendel, C.R.; Kral-O'Brien, K.C.; Hovick, T.J.; Limb, R.F.; Harmon, J.P. Plant–Pollinator Networks in Grassland Working Landscapes Reveal Seasonal Shifts in Network Structure and Composition. *Ecosphere* **2019**, *10*, e02569. [CrossRef]
20. Burlec, A.F. Phytochemical Composition and in vitro Biological Properties of Several *Rudbeckia hirta* and *Tagetes erecta* Flower Extracts. *FARMACIA* **2022**, *70*, 241–247. [CrossRef]
21. Hopkins, J.R.; Bennet, A.E. Fire Effects on Soil Biota Alter the Strength and Direction of Plant-Soil Feedbacks between *Schizachyrium scoparium* (Michx.) Nash and *Rudbeckia hirta* L. *Plant Soil* **2025**, *517*, 25–39. [CrossRef]
22. Yan, A.; Chen, Z. The Control of Seed Dormancy and Germination by Temperature, Light and Nitrate. *Bot. Rev.* **2020**, *86*, 39–75. [CrossRef]
23. Yang, L.; Liu, S.; Lin, R. The Role of Light in Regulating Seed Dormancy and Germination. *J. Integr. Plant Biol.* **2020**, *62*, 1310–1326. [CrossRef] [PubMed]
24. Fraser, L.H.; Mulac, K.; Moore, F.B.-G. Germination of 14 Freshwater Wetland Plants as Affected by Oxygen and Light. *Aquat. Bot.* **2014**, *114*, 29–34. [CrossRef]
25. Ermiş, S.; Amirkhani, M.; Loos, M.T.; Taylor, A.G. Investigations into Selected Pollinator-Friendly Plant Species: Seed Lot Germination, Breaking Dormancy with Plant Hormone Priming and the Influence of Water Stress on Germination. *Horticulturae* **2025**, *12*, 32. [CrossRef]
26. Adedeji-Badmus, A.N.; Schramm, S.; Gigl, M.; Iwebema, W.; Albertos, P.; Dawid, C.; Sieberer, T.; Poppenberger, B. Species-Specific Variation in Abscisic Acid Homeostasis and Responses Impacts Important Traits in *Crassocephalum* Orphan Crops. *Front. Plant Sci.* **2022**, *13*, 923421. [CrossRef] [PubMed]
27. Sari, A.O.; Tutar, M. Effects of Light, Cold Storage, and Temperature on Seed Germination of Golden Thistle (*Scolymus hispanicus* L.). *J. Herbs Spices Med. Plants* **2010**, *15*, 318–325. [CrossRef]
28. Pliszko, A.; Kostrakiewicz-Gierałt, K. Effect of Cold Stratification on Seed Germination in *Solidago* × *Niederederi* (Asteraceae) and Its Parental Species. *Biologia* **2018**, *73*, 945–950. [CrossRef] [PubMed]
29. Austin, M.W.; Smith, A.B.; Olsen, K.M.; Hoch, P.C.; Krakos, K.N.; Schmocker, S.P.; Miller-Struttmann, N.E. Climate Change Increases Flowering Duration, Driving Phenological Reassembly and Elevated Co-flowering Richness. *New Phytol.* **2024**, *243*, 2486–2500. [CrossRef] [PubMed]
30. Craig, D.S.; Runkle, E.S. An Intermediate Phytochrome Photoequilibria from Night-Interruption Lighting Optimally Promotes Flowering of Several Long-Day Plants. *Environ. Exp. Bot.* **2016**, *121*, 132–138. [CrossRef]
31. Gahory, A.A.; Salah Edeen, R.; Mahmoud, A.; Abdel-Mola, M. Effect of Organic and Bio Fertilization on the Growth and Flowering of *Rudbeckia hirta* Plants. *Sci. J. Agric. Sci.* **2022**, *4*, 40–49. [CrossRef]
32. Mills-Ibibofori, T.; Dunn, B.; Maness, N.; Payton, M. Use of Diatomaceous Earth as a Silica Supplement on Potted Ornamentals. *Horticulturae* **2019**, *5*, 21. [CrossRef]
33. Stahlhut, K.N.; Neupert, D.G.; Laing, J.E.; Witt, L.J.; Bauer, J.T. Measuring Leaf and Root Functional Traits Uncovers Multidimensionality of Plant Responses to Arbuscular Mycorrhizal Fungi. *Am. J. Bot.* **2024**, *111*, e16369. [CrossRef] [PubMed]
34. Costa, B.N.S.; Tucker, D.A.; Khoddamzadeh, A.A. Precision Horticulture: Application of Optical Sensor Technology for Nitrogen Monitoring Status in Cocoplum, a Native Landscaping Plant. *Plants* **2023**, *12*, 760. [CrossRef] [PubMed]
35. Khoddamzadeh, A.A.; Poudel, I.; Jayachandran, K. Sensor-Based Nutrient Diagnostics for Optimizing Nitrogen Management in Poinsettia 'Bravo Bright Red'. *HortScience* **2026**, *61*, 1318–1326. [CrossRef]

36. Nogueira Souza Costa, B.; Khoddamzadeh, A.A. Data-Driven Nitrogen Application for Satinleaf: Leveraging Optical Sensors in Urban Landscape Management. *Front. Plant Sci.* **2025**, *16*, 1522662. [CrossRef] [PubMed]
37. Klupczyńska, E.A.; Pawłowski, T.A. Regulation of Seed Dormancy and Germination Mechanisms in a Changing Environment. *Int. J. Mol. Sci.* **2021**, *22*, 1357. [CrossRef] [PubMed]
38. Hasnain, A.; Naqvi, S.A.H.; Ayesha, S.I.; Khalid, F.; Ellahi, M.; Iqbal, S.; Hassan, M.Z.; Abbas, A.; Adamski, R.; Markowska, D.; et al. Plants in vitro Propagation with Its Applications in Food, Pharmaceuticals and Cosmetic Industries; Current Scenario and Future Approaches. *Front. Plant Sci.* **2022**, *13*, 1009395. Erratum in *Front. Plant Sci.* **2023**, *14*, 1197747. [CrossRef] [PubMed]
39. Mehbub, H.; Akter, A.; Akter, M.A.; Mandal, M.S.H.; Hoque, M.A.; Tuleja, M.; Mehraj, H. Tissue Culture in Ornamentals: Cultivation Factors, Propagation Techniques, and Its Application. *Plants* **2022**, *11*, 3208. [CrossRef] [PubMed]
40. Gautam, N.; Faroda, P.; Agarwal, T.; Harish; Gupta, A.K. Effect of Microclimatic Physical Factors on in vitro Morphogenesis of Plants: A Systematic Review. *Discov. Agric.* **2024**, *2*, 13. [CrossRef]
41. Mosoh, D.A.; Khandel, A.K.; Verma, S.K.; Vendrame, W.A. Overcoming Dual Seed Dormancy and Enhancing in vitro Seedling Development of *Gloriosa superba* (L.) with a Targeted Sterilization Approach and Plant Growth Regulator Synergy. *Trop. Plants* **2024**, *3*, e031. [CrossRef]
42. Tinak Ekom, D.C.; Lombeko Obe Tomo, V.; Mongoue Fanche, A.; Signi, C.I.; Sitcha Tchouatcha, N.G.; Sontsa-Donhoung, A.M.; Mahot, H.C.; Mapiemfu-Lamare, D.; Bougnom, B.P. Genotype-Specific Disinfection Protocols Enable Reliable in vitro Culture from Mature Field-Harvested Maize Seeds. *Discov. Plants* **2026**, *3*, 90. [CrossRef]
43. Sun, Y.; Han, L.; Zhang, D. In vitro Regeneration of *Rudbeckia hirta* 'Plainview Farm'from Leaf Tissue. *Internatioal J. Biochem. Biotechnol.* **2012**, *1*, 33.
44. Kildisheva, O.A.; Erickson, T.E.; Madsen, M.D.; Dixon, K.W.; Merritt, D.J. Seed Germination and Dormancy Traits of Forbs and Shrubs Important for Restoration of North American Dryland Ecosystems. *Plant Biol.* **2019**, *21*, 458–469. [CrossRef] [PubMed]
45. Farias, K.S.; Ferreira, M.M.; De Oliveira, I.B.; Dalio, R.J.D.; Pirovani, C.P. The BASIDIN Effector of the Fungus Moniliophthora Perniciosa Promotes Positive Effects on the Seed Germination and Seedlings Development of Lactuca Sativa. *Front. Plant Sci.* **2025**, *16*, 1529096. [CrossRef] [PubMed]
46. Lozano-Isla, F.; Benites-Alfaro, O.E.; Pompelli, M.F. GerminaR: An R Package for Germination Analysis with the Interactive Web Application "GerminaQuant for R.". *Ecol. Res.* **2019**, *34*, 339–346. [CrossRef]
47. Izarra, M.L.; Panta, A.L.; Maza, C.R.; Zea, B.C.; Cruzado, J.; Gutarra, L.R.; Rivera, C.R.; Ellis, D.; Kreuze, J.F. Identification and Control of Latent Bacteria in in vitro Cultures of Sweetpotato [*Ipomoea batatas* (L.) Lam]. *Front. Plant Sci.* **2020**, *11*, 903. [CrossRef] [PubMed]
48. Abdalla, N.; El-Ramady, H.; Seliem, M.K.; El-Mahrouk, M.E.; Taha, N.; Bayoumi, Y.; Shalaby, T.A.; Dobránszki, J. An Academic and Technical Overview on Plant Micropropagation Challenges. *Horticulturae* **2022**, *8*, 677. [CrossRef]
49. Darçın, E.S.; Kolsarıcı, Ö.; Yıldız, M. Establishment of Efficient Regeneration Protocol for Three Rapeseed Cultivars. *Biotechnol. Biotechnol. Equip.* **2014**, *28*, 21–26. [CrossRef] [PubMed]
50. Mendes, F.M.; Leite, D.M.; De Sousa, K.I.R.; Fernandes, S.B.; Brondani, G.E. Sterilization and Gas Exchange on in vitro Cultivation of *Sinningia rupicola* (Mart.) Wiehler: Production of Seedlings from an Endangered Species. *Vitro Cell. Dev. Biol. Plant* **2026**, *62*, 740–751. [CrossRef]
51. Ozel, C.A.; Khawar, K.M.; Mirici, S.; Ozcan, S.; Arslan, O. Factors Affecting in vitro Plant Regeneration of the Critically Endangered Mediterranean Knapweed (*Centaurea tchihatcheffii* Fisch et. Mey). *Naturwissenschaften* **2006**, *93*, 511–517. [CrossRef] [PubMed]
52. Sugii, N.C. The Establishment of Axenic Seed and Embryo Cultures of Endangered Hawaiian Plant Species: Special Review of Disinfestation Protocols. *Vitro Cell. Dev. Biol. Plant* **2011**, *47*, 157–169. [CrossRef]
53. Barampuram, S.; Allen, G.; Krasnyanski, S. Effect of Various Sterilization Procedures on the in vitro Germination of Cotton Seeds. *Plant Cell Tissue Organ Cult. PCTOC* **2014**, *118*, 179–185. [CrossRef]
54. Munoz-Salas, M.N.; Nageswara-Rao, M.; Khoddamzadeh, A.A. Optimization of Sterilization Strategies and Hormonal Regulation for in vitro Establishment and Early Regeneration of *Musa haekkinenii*. *HortTechnology* **2026**, *36*, 341–351. [CrossRef]
55. Lozano-Isla, F.; Farfan-Vignolo, E.R.; Gutierrez, R.; Blas, R.; Awais, K. Harvest Index Is a Key Trait for Screening Drought-Tolerant Potato Genotypes (*Solanum tuberosum*). *J. Crop Sci. Biotechnol.* **2024**, *27*, 91–103. Correction to *J. Crop Sci. Biotechnol.* **2025**, *28*, 153. [CrossRef]
56. R Core Team. *R: A Language and Environment for Statistical Computing 2026*; R Foundation for Statistical Computing: Vienna, Austria, 2026.
57. Lozano-Isla, F.; Diaz-Saucedo, Y. Inti: Tools and Statistical Procedures in Plant Science 2020, Version 0.7.2. Available online: https://cran.r-project.org/web/packages/inti/index.html (accessed on 15 October 2026).
58. Piepho, H.-P.; Gabriel, D.; Hartung, J.; Büchse, A.; Grosse, M.; Kurz, S.; Laidig, F.; Michel, V.; Proctor, I.; Sedlmeier, J.E.; et al. One, Two, Three: Portable Sample Size in Agricultural Research. *J. Agric. Sci.* **2022**, *160*, 459–482. [CrossRef]
59. Bates, D.; Maechler, M.; Bolker, B.; Walker, S.; Ly, A.; Jagan, M. Lme4: Linear Mixed-Effects Models Using "Eigen" and S4 2003, Version 2.0-6. Available online: https://cran.r-project.org/web/packages/lme4/index.html (accessed on 15 October 2026).
60. Fox, J.; Weisberg, S.; Price, B. Car: Companion to Applied Regression 2001, Version 3.1-5. Available online: https://cran.r-project.org/web/packages/car/index.html (accessed on 15 October 2026).

61. Lenth, R.V.; Piaskowski, J. Emmeans: Estimated Marginal Means, Aka Least-Squares Means 2017, Version 2.0.4. Available online: https://cran.r-project.org/web/packages/emmeans/index.html (accessed on 15 October 2026).
62. Husson, F.; Josse, J.; Le, S.; Mazet, J. FactoMineR: Multivariate Exploratory Data Analysis and Data Mining 2006, Version 2.16. Available online: https://cran.r-project.org/web/packages/FactoMineR/index.html (accessed on 15 October 2026).
63. Wickham, H.; Chang, W.; Henry, L.; Pedersen, T.L.; Takahashi, K.; Wilke, C.; Woo, K.; Yutani, H.; Dunnington, D.; Van Den Brand, T. Ggplot2: Create Elegant Data Visualisations Using the Grammar of Graphics 2007, Version 4.0.3. Available online: https://cran.r-project.org/web/packages/ggplot2/index.html (accessed on 15 October 2026).
64. Wickham, H. Tidyverse: Easily Install and Load the "Tidyverse" 2016, Version 2.0.0. Available online: https://cran.r-project.org/web/packages/tidyverse/index.html (accessed on 15 October 2026).
65. Hoffman, G.R. Germination of Herbaceous Plants Common to Aspen Forests of Western Colorado. *Bull. Torrey Bot. Club* **1985**, *112*, 409. [CrossRef]
66. Ferreira, A.G.; Cassol, B.; Rosa, S.G.T.D.; Silveira, T.S.D.; Stival, A.L.; Silva, A.A. Germinação de Sementes de Asteraceae Nativas No Rio Grande Do Sul, Brasil. *Acta Bot. Bras.* **2001**, *15*, 231–242. [CrossRef]
67. Trotta, G.; Vuerich, M.; Petrussa, E.; Hay, F.R.; Assolari, S.; Boscutti, F. Germination Performance of Alien and Native Species Could Shape Community Assembly of Temperate Grasslands under Different Temperature Scenarios. *Plant Ecol.* **2023**, *224*, 1097–1111. [CrossRef]
68. Schutz, W. Seed Dormancy, After-Ripening and Light Requirements of Four Annual Asteraceae in South-Western Australia. *Ann. Bot.* **2002**, *90*, 707–714. [CrossRef] [PubMed]
69. De Queiroz, A.R.; Maricle, B.R. Effects of Light Levels on Germination of Five Asteraceae Species Native to the Tallgrass Prairie. *Trans. Kans. Acad. Sci.* **2019**, *122*, 267–273. [CrossRef]
70. Baek, J.J.; Lee, Y.J.; Nam, G.-H.; Kang, J.-W.; Lee, S.Y. Non-Deep Physiological Dormancy in Seeds of *Crepidiastrum denticulatum* (Houtt.) J.H.Pak & Kawano. *Agronomy* **2025**, *16*, 23. [CrossRef]
71. Rocchetti, G.A.; Brancaleoni, L.; Caneva, G.; Cona, A.; Fabrini, G.; Fraudentali, I.; Galasso, G.; Godefroid, S.; Iberite, M.; Lastrucci, L.; et al. Testing Seed Germination from Herbaria: Application of Seed Quality Enhancement Techniques and Implication for Plant Resurrection and Conservation. *Taxon* **2024**, *73*, 854–867. [CrossRef]
72. Gammoudi, N.; Nagaz, K.; Ferchichi, A. Establishment of Optimized in vitro Disinfection Protocol of *Pistacia Vera* L. Explants Mediated a Computational Approach: Multilayer Perceptron–Multi−objective Genetic Algorithm. *BMC Plant Biol.* **2022**, *22*, 324. [CrossRef] [PubMed]
73. Si, Y.; Haxim, Y.; Wang, L. Optimum Sterilization Method for in vitro Cultivation of Dimorphic Seeds of the Succulent Halophyte *Suaeda aralocaspica*. *Horticulturae* **2022**, *8*, 289. [CrossRef]
74. Gijsman, F.; Vitt, P. Seed Size and Capitulum Position Drive Germination and Dormancy Responses to Projected Warming for the Threatened Dune Endemic *Cirsium pitcheri* (Asteraceae). *Ecol. Evol.* **2021**, *11*, 955–966. [CrossRef] [PubMed]
75. Bošnjak Mihovilović, A.; Kereša, S.; Lazarević, B.; Topolovec Pintarić, S.; Martinko, K.; Marković, Z.; Turkalj, K.; Habuš Jerčić, I. The Use of Sodium Hypochlorite and Plant Preservative Mixture Significantly Reduces Seed-Borne Pathogen Contamination When Establishing in vitro Cultures of Wheat (*Triticum aestivum* L.) Seeds. *Agriculture* **2024**, *14*, 556. [CrossRef]
76. Rihan, H.Z.; Al-Issawi, M.; Al-swedi, F.; Fuller, M.P. The Effect of Using PPM (Plant Preservative Mixture) on the Development of Cauliflower Microshoots and the Quality of Artificial Seed Produced. *Sci. Hortic.* **2012**, *141*, 47–52. [CrossRef]
77. Ma, D.; Yi, B.; Teng, W.; Ali, I.; Shao, J.; Lin, Y.; Yu, J.; Tian, X.; Wang, Y.; Wang, L. Growth, Physiological and N, P, K Accumulation Responses of *Erythropalum scandens* Bl. Seedlings under Different Substrates. *BMC Plant Biol.* **2024**, *24*, 972. [CrossRef] [PubMed]
78. Bu, X.; Ji, H.; Ma, W.; Mu, C.; Xian, T.; Zhou, Z.; Wang, F.; Xue, J. Effects of Biochar as a Peat-Based Substrate Component on Morphological, Photosynthetic and Biochemical Characteristics of *Rhododendron delavayi* Franch. *Sci. Hortic.* **2022**, *302*, 111148. [CrossRef]
79. Di Lonardo, S.; Cacini, S.; Becucci, L.; Lenzi, A.; Orsenigo, S.; Zubani, L.; Rossi, G.; Zaccheo, P.; Massa, D. Testing New Peat-Free Substrate Mixtures for the Cultivation of Perennial Herbaceous Species: A Case Study on *Leucanthemum vulgare* Lam. *Sci. Hortic.* **2021**, *289*, 110472. [CrossRef]
80. Rasool, S.; Ahmad, I.; Ziaf, K.; Naveed, M. Plant Growth Promoting Rhizobacteria Incorporated Soilless Substrates—A Potential Arena for High Quality Nursery Production of Ornamentals. *Sci. Hortic.* **2024**, *326*, 112745. [CrossRef]
81. Carapezza, G.; Wilson, S.B.; McMillan, M.; Thetford, M. Effects of Container Substrate Composition on the Growth and Performance of *Garberia heterophylla* (W. Bartram) Merr. & F. Harper: A Native Xeric Species. *Horticulturae* **2025**, *11*, 982. [CrossRef]
82. Costa, B.N.S.; Salas, M.N.M.; Gil, K.; Khoddamzadeh, A.A. Effect of Silicon Amendment on Growth and Nitrogen Status of Common Landscaping Plants. *Am. J. Plant Sci.* **2024**, *15*, 603–616. [CrossRef]
83. Yang, T.; Altland, J.E.; Samarakoon, U.C. Evaluation of Substrates for Cucumber Production in the Dutch Bucket Hydroponic System. *Sci. Hortic.* **2023**, *308*, 111578. [CrossRef]
84. Ali, A.M.; Ibrahim, S.M.; Bijay-Singh. Wheat Grain Yield and Nitrogen Uptake Prediction Using atLeaf and GreenSeeker Portable Optical Sensors at Jointing Growth Stage. *Inf. Process. Agric.* **2020**, *7*, 375–383. [CrossRef]

85. Markwell, J.; Osterman, J.C.; Mitchell, J.L. Calibration of the Minolta SPAD-502 Leaf Chlorophyll Meter. *Photosynth. Res.* **1995**, *46*, 467–472. [CrossRef] [PubMed]
86. Khoddamzadeh, A.A.; Dunn, B.L. Application of Optical Sensors for Nitrogen Management in Chrysanthemum. *HortScience* **2016**, *51*, 915–920. [CrossRef]
87. Mendoza-Tafolla, R.O.; Juarez-Lopez, P.; Ontiveros-Capurata, R.-E.; Sandoval-Villa, M.; Alia-Tejacal, I.; Alejo-Santiago, G. Estimating Nitrogen and Chlorophyll Status of Romaine Lettuce Using SPAD and at LEAF Readings. *Not. Bot. Horti Agrobot. Cluj. Napoca* **2019**, *47*, 751–756. [CrossRef]
88. Dunn, B.L.; Shrestha, A.; Goad, C. Determining Nitrogen Fertility Status Using Optical Sensors in Geranium with Controlled Release Fertilizer. *J. Appl. Hortic.* **2015**, *17*, 7–11. [CrossRef]
89. Burlec, A.F.; Pecio, Ł.; Mircea, C.; Tuchiluș, C.; Corciovă, A.; Danciu, C.; Cioancă, O.; Caba, I.C.; Pecio, S.; Oleszek, W.; et al. Preliminary Phytochemical and Biological Evaluation of *Rudbeckia hirta* Flowers. *Plants* **2023**, *12*, 2871. [CrossRef] [PubMed]

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