A Comparative Study of the Influence of Soil and Non-Soil Factors on Seed Germination of Edible Salt-Tolerant Species

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Abstract: Cultivating edible salt-tolerant plants (halophytes) for human consumption is increasingly important due to climate change and soil salinization, and offers sustainable agricultural solutions. Optimizing seed germination, the crucial initial stage of crop growth, is essential for enhancing crop production. This study aimed to optimize the germination of edible halophytes under greenhouse conditions, focusing on select soil (salinity and substrate) and non-soil-related factors (chemical and mechanical treatments). The target species were selected for their commercial value and included Mesembryanthemum crystallinum L. (crystalline iceplant), Salicornia ramosissima J. Woods (sea asparagus), Medicago marina L. (sea medick), Ammophila arenaria (L.) Link (European beachgrass), Portulaca oleracea L. (common purslane), and Atriplex halimus L. (Mediterranean saltbush). Salinity negatively impacted germination rates (GRs) and delayed mean germination time (MGT) across species. P. oleracea had the highest GR (95.6%) in coco peat under freshwater irrigation, and the shortest MGT (5.2 days). A. halimus did not germinate under the tested conditions. Scarification with sulfuric acid improved the GR of M. marina by 42.2%, while scarification with ultrasounds improved the GR of A. arenaria by 35.5%. Our results indicate that the choice of substrate and the application of specific treatments like scarification can significantly improve the germination of certain halophyte species under variable saline conditions.

Keywords: climate change; halophytes; salinization; saline agriculture; sustainability

1. Introduction

Freshwater is a scarce resource in most Mediterranean countries, primarily due to the agricultural sector, which accounts for 70% of global freshwater withdrawals [1,2]. Additionally, water salinization is an emerging global problem caused by anthropogenic factors such as climate change, sea-level rise, urbanization, and agriculture, as well as natural causes like tidal influences and seasonal cycles [3]. Soil salinization affects nearly 20% of cultivated land worldwide, severely limiting arable land area, reducing crop productivity, and threatening agricultural sustainability and food security [4]. Salt stress decreases the productivity of most commercial crops, restricting the necessary increase in food crop production to meet the demands of a growing population, estimated to reach an additional 2.3 billion people by 2050 [5]. To address these challenges, efforts have been made to develop more resistant crops, including of resistant cultivars, and the recruitment of salt-tolerant plant species with agronomic potential. Furthermore, achieving the United Nations Sustainable Development Goals (SDG) 2 for food security and SDG 6 for water security within the framework of the water–energy–food–ecosystem nexus necessitates a transition to diets that are both nutritious and sustainable [6,7].

Salt-tolerant plants, or halophytes, thrive in habitats with stressful climatic conditions, such as salt marshes, inland deserts, salt flats, and steppes, and can withstand soil and water salt concentrations of at least 200 mmol·L⁻¹ [8]. Halophytes possess high biotechnological potential due to their chemical composition, which includes nutritional elements...
(e.g., minerals and fiber) and secondary metabolites (e.g., phenolic acids and flavonoids) with health-improving properties (e.g., antioxidant and anti-diabetic) [9,10]. Historically, halophytes have been used worldwide for food and therapeutic purposes [11], and today, several species are considered gourmet foods, including quinoa seeds (*Chenopodium quinoa* Willd.), iceplant (*Mesembryanthemum crystallinum* L.), and sea asparagus (*Sarcocornia* and *Salicornia* spp.) [12–14]. Halophytes can be cultivated in a variety of saline conditions, including marginal saline soils and greenhouse aquaponics. They hold significant economic potential as sources of food products, bioactive products with health-improving properties, animal feed, and biofuel feedstocks, while contributing to soil phytoremediation and coastal protection efforts [15]. Therefore, recruiting new species and optimizing the cultivation of halophytic species for commercial purposes is a valuable strategy to address soil and water salinization, improve growth conditions, ensure a consistent supply of nutrients, and produce high-quality, safe food products [9,16].

Seed germination is the first stage of crop growth in the plant life cycle and plays a crucial role in subsequent crop production, making it the primary stage to optimize. The germination of halophyte seeds is influenced by various environmental factors, including salinity, light, and temperature, and is species-dependent [17]. Some species exhibit reduced germination rates and significant seed dormancy, which can be overcome, for example, through the use of chemical treatments (e.g., gibberellic acid and proline) applied to *Halogeton glomeratus* (M.Bieb.) Ledeb., *Lepidium latifolium* L., and *Peganum harmala* L. [18], and physical treatments (e.g., using a 500 μm sieve and a pestle) in *Salicornia herbacea* L. [19]. Soil physical properties also affect germination, as a suitable substrate should offer optimal water availability for seed germination [20]. These properties include porosity, volume, and density of the substrate, as well as water availability for the plant [21]. There is no universal optimum substrate or mixture for all species and cultivation processes. Examples include a mixture of peat and perlite (3:1, v/v) used in the cultivation of *Limonium algarvense* Erben [22], vermicompost for *Suaeda salsa* (L.) Pall. [23], and a mixture of clay and sand for *Salicornia dolichostachya* Moss and *S. brachystachya* (GFW Meyer) König [24].

In our ongoing efforts to optimize the sustainable saline cultivation of edible halophytes, this research focused on the influence of soil-related factors (salinity and substrate) and non-soil-related factors (chemical and mechanical treatments) on the germination of seeds of six halophytic species in greenhouse conditions. The target species included *Mesembryanthemum crystallinum* L. (crystalline iceplant), *Salicornia ramosissima* J. Woods (sea asparagus), *Medicago marina* L. (sea medick), *Ammophila arenaria* (L.) Link (European beachgrass), *Portulaca oleracea* L. (common purslane), and *Atriplex halimus* L. (Mediterranean saltbush). These species were selected for their commercial interest due to their traditional uses, and nutritional and functional properties, as outlined in Table S1 (Supplementary Materials). There are few studies that have focused on the best germination conditions for the species included in this work. These studies include examining the effects of light treatments on the growth and chemical composition of *M. crystallinum* [25], investigating the impact of seed dimorphism and salinity [26], as well as bacterial inoculation [27], on the germination efficiency of *S. ramosissima*, and exploring mechanisms controlling dormancy and germination in *M. marina* [28]. Additionally, research has determined the optimal germination temperature for *A. arenaria* [29] and evaluated various factors affecting germination in *P. oleracea* [30–32] and *A. halimus* [33–35], such as site of origin, seed maturation time, seed age, salinity, and temperature. However, such studies have largely focused on germination under controlled laboratory settings, and, therefore, the practical application of these findings to commercial-scale production remains limited. Assessing the germination of edible halophytes under greenhouse conditions is critical for advancing sustainable agriculture in saline environments, since greenhouse environments offer a more realistic intermediate between laboratory and field conditions, incorporating natural variability while maintaining some control over environmental factors. Therefore, this work aims to bridge the gap by developing germination protocols that are not only scientifically robust but also scalable and applicable to commercial farming. By doing so, it addresses the urgent
need for sustainable crop production methods in the face of increasing soil salinization and global food demand.

2. Materials and Methods

2.1. Seed Acquisition and Greenhouse Conditions

Seeds were purchased from seed commercial suppliers: *S. ramosissima* from Horta da Ria Lda. (Aveiro, Portugal) and *M. crystallinum, M. marina, A. arenaria* and *A. halimus*, and *P. oleracea* from Semillas Cantueso S.L., (Córdoba, Spain), stored in dry conditions in darkness at room temperature (RT, about 20 °C), and used in the assays in the same year of their collection. Experiments were conducted throughout April–June 2021 in a greenhouse located in the University of Algarve (Faro, Portugal). The average air temperature was 24.8 °C, with a minimum and maximum of 12.8 °C and 45.3 °C, respectively, and an average relative air humidity (RH) of 52.2%, with a minimum and maximum of 21.5% and 78.9%, correspondingly. Seeds were sown in styrofoam trays of 198 cavities (53 mm deep and 22.05 cm³ cell capacity) filled with the corresponding substrate and water irrigation according to the experiment. All species were subjected to the same germination conditions.

2.2. Germination Conditions

Four replicates of 40 seeds each were sown in the corresponding substrate and irrigation water conditions. The following substrates were tested: perlite, vermiculite, coco peat, sand, coco peat and perlite (1:1 v/v), coco peat and vermiculite (1:1 v/v), perlite and vermiculite (1:1 v/v), and sand, organic peat and perlite (1:1:1 v/v/v). The salinity of the irrigation water was evaluated by using fresh water and saline water (from a well, conductivity of 20.1 mS/cm). Germination was monitored twice a week for 28 days, and seeds were considered germinated when the radicle emerged from the substrate. Two indices were used to evaluate germination: (1) the final germination percentage, expressed as germination rate (GR) and calculated as the ratio between the total number of germinated seeds and the total number of seeds, multiplied by 100, and (2) the mean germination time (MGT), calculated by Equation (1) [36]:

\[ \text{MGT} = \frac{\sum (n \times d)}{N} \] (1)

where n = number of seeds germinated in each interval; d = incubation period at that time point (in days); and N = total number of germinated seeds.

2.3. Seed Treatments

Four replicates of 20 seeds each of species with a GR lower than 50% were submitted to five treatments, as follows: (1) chemical treatment—seeds were soaked in gibberellic acid (1 g/L; Merck, Darmstadt, Germany) for 24 h at RT (approx. 20 °C); (2) chemical scarification—seeds were soaked in sulfuric acid (50%; Merck, Darmstadt, Germany) for 10 min at RT; (3) mechanical scarification—seeds were soaked in distilled water and placed for 15 min in an ultrasonic water bath (USC-TH, VWR, Darmstadt, Germany) at RT; (4) water soaking—seeds were soaked in distilled water for 24 h at RT; and (5) thermal shock—seeds were soaked in distilled water at different temperatures, namely 60 °C for 10 min and 24 h in water (W60), 75 °C for 20 min (W75), 75 °C for 20 min and at −4 °C for 20 min (W75W-4), −4 °C for 20 min and 75 °C for 20 min (W-4W75), and −4 °C for 20 min (W-4). After treatment, seeds were sown in the conditions of water salinity and substrate that allowed for the highest GR. Germination was monitored twice a week for 28 days and seeds were considered germinated when the radicle emerged from the substrate.

2.4. Statistical Analysis

Results were expressed as mean ± standard error of the mean (SEM). A two-way ANOVA was used to test the significance of substrate, irrigation, and their interaction on GR and MGT, and a one-way ANOVA was used to test the significance of specific treatments to improve the germination of species with a GR lower than 50%. Tukey’s HSD
test were performed to determine significant ($p < 0.05$) differences between individual treatments. A principal component analysis (PCA) was performed using the Pearson correlation coefficient ($r$) ($p < 0.05$) as an index of similarity. All the results were analyzed using the XLSTAT statistical package (v.2015.6.01.23865, Addinsoft, New York, NY, USA).

3. Results and Discussion

As can be seen in Table 1, GRs and MGTs varied significantly among species and cultivation conditions (one-way ANOVA, Tables S2 and S3 of the Supplementary Materials). *P. oleracea* showed the highest GR (95.6%) and the shortest MGT (5.2 days) in coco peat under freshwater irrigation, following previous findings in the same species where experiments occurred in petri dishes in controlled conditions (germination chamber) [31]. Other species showed varying GRs and MGTs depending on the substrate and salinity; for example, *M. marina* showed the best GR (11.1%) in vermiculite and *A. arenaria* (16.7%) in coco peat, both under freshwater irrigation. *M. crystallinum* reached the highest GR in a coco peat and vermiculite mix under freshwater irrigation (GR, 52.2%; MGT, 8.44 days). Similar results were obtained for *M. crystallinum* under controlled conditions in a growth chamber with different photoperiods and light intensities, cultivated in sand irrigated with freshwater and supplemented with a nutrient solution (GR, 53.75%; MGT, 3.70 days) [25]. In the case of *S. ramosissima*, the highest GR was reached in coco peat and vermiculite under freshwater irrigation (GR: 66.7%; MGT: 13.2 days). In a previous study, *S. ramosissima* seeds were germinated in petri dishes under controlled conditions and exposed to irrigation solutions with increasing salinity, which resulted in a decrease in GR of 15% for salinities higher than 342 mmolL$^{-1}$ of NaCl [26]. *M. crystallinum*, *M. marina*, and *A. arenaria* did not germinate under saltwater irrigation, which may be explained by osmotic stress, ionic toxicity, and plant adaptation to seed recollection site [37,38]. Germination response depends, to some extent, on geographic variation in environmental conditions from the original seed collection site [38]. Plants exposed to adverse salinity conditions will develop greater resilience, enabling them to thrive in extreme environments, as in the case of *C. quinoa* at 500 mmolL$^{-1}$ of NaCl with seeds from salt flats (GR about 45%) and coastal lowlands (GR about 20%) [38].

*A. halimus* did not germinate under the tested conditions, and, therefore, seed viability was checked by the tetrazolium test, an established method to score seed quality [39], revealing that not all seeds were viable. Seeds with low or nil viability are common and can be caused by different factors, including storage conditions (length and conditions of storage), the presence of incomplete embryos, and the absence of embryos [40]. *A. halimus* is a xerophytic halophyte that grows mainly in dry saline soil [41], so seeds soaked in fresh or seawater could be non-optimal conditions and the seeds would be in a dormant state, as occurs in other species of the *Atriplex* genus, such as *A. confertifolia* (Torr. & Frem.) Wats and *A. sagittata* Borkh [42,43]. Moreover, the period between seed collection and the assays (seven months) may have influenced seed germination. Research on seed storage time has shown that seeds can lose their viability after a period of 3 months for *Tamarix* species, 12 months for *Arthrocnemum indicum*, and 14 months for *Cressa cretica* [44,45]. In a previous study on the same species, a GR of 55% was achieved in seeds after 33 days in storage when germination occurred under controlled conditions under freshwater irrigation, decreasing by 10–15% after 272 days in storage [34]. ROS are continuously produced during seed storage, and, therefore, may be implicated in the loss of viability by causing oxidative damage during seed imbibition [46].
Table 1. Germination rates (GRs, %) and mean germination times (MGTs) of seeds of M. crystallinum, S. ramosissima, M. marina, A. arenaria, P. oleracea, and A. halimus under greenhouse cultivation.

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Irrigation</th>
<th>M. crystallinum</th>
<th>S. ramosissima</th>
<th>M. marina</th>
<th>A. arenaria</th>
<th>P. oleracea</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>GR</td>
<td>MGT</td>
<td>GR</td>
<td>MGT</td>
<td>GR</td>
</tr>
<tr>
<td>Perlite</td>
<td>Freshwater</td>
<td>43.3 ± 1.5 a</td>
<td>6.3 a</td>
<td>52.2 ± 1.5 abc</td>
<td>9.2 b</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Saltwater</td>
<td>-</td>
<td>-</td>
<td>5.6 ± 0.3 a</td>
<td>9.4 ab</td>
<td>-</td>
</tr>
<tr>
<td>Vermiculite</td>
<td>Freshwater</td>
<td>35.6 ± 3.2 a</td>
<td>6.0 a</td>
<td>50.0 ± 1.5 abc</td>
<td>12.2 ab</td>
<td>11.1 ± 0.7 a</td>
</tr>
<tr>
<td></td>
<td>Saltwater</td>
<td>-</td>
<td>-</td>
<td>14.4 ± 0.9 de</td>
<td>12.6 ab</td>
<td>-</td>
</tr>
<tr>
<td>Coco peat</td>
<td>Freshwater</td>
<td>40.0 ± 2.1 a</td>
<td>6.3 a</td>
<td>57.8 ± 1.8 abc</td>
<td>14.2 ab</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Saltwater</td>
<td>-</td>
<td>-</td>
<td>10.0 ± 1.2 de</td>
<td>14.5 ab</td>
<td>-</td>
</tr>
<tr>
<td>Sand</td>
<td>Freshwater</td>
<td>35.6 ± 2.2 a</td>
<td>10.6 a</td>
<td>57.8 ± 0.9 abc</td>
<td>16.7 ab</td>
<td>5.6 ± 0.3 ab</td>
</tr>
<tr>
<td></td>
<td>Saltwater</td>
<td>-</td>
<td>-</td>
<td>45.6 ± 2.0 abc</td>
<td>17.1 ab</td>
<td>-</td>
</tr>
<tr>
<td>Coco peat, perlite (1:1 v/v)</td>
<td>Freshwater</td>
<td>38.9 ± 1.5 a</td>
<td>6.2 a</td>
<td>35.6 ± 1.2 bcd</td>
<td>11.5 ab</td>
<td>6.7 ± 0.5 ab</td>
</tr>
<tr>
<td></td>
<td>Saltwater</td>
<td>-</td>
<td>-</td>
<td>31.1 ± 2.1 cde</td>
<td>12.9 ab</td>
<td>-</td>
</tr>
<tr>
<td>Coco peat, vermiculite (1:1 v/v)</td>
<td>Freshwater</td>
<td>52.2 ± 2.2 a</td>
<td>8.4 a</td>
<td>66.7 ± 2.6 a</td>
<td>13.2 ab</td>
<td>2.2 ± 0.3 b</td>
</tr>
<tr>
<td></td>
<td>Saltwater</td>
<td>-</td>
<td>-</td>
<td>33.3 ± 2.1 cde</td>
<td>17.5 a</td>
<td>-</td>
</tr>
<tr>
<td>Perlite, vermiculite (1:1 v/v)</td>
<td>Freshwater</td>
<td>45.6 ± 1.2 a</td>
<td>6.0 a</td>
<td>57.8 ± 0.7 abc</td>
<td>10.4 ab</td>
<td>4.4 ± 0.3 b</td>
</tr>
<tr>
<td></td>
<td>Saltwater</td>
<td>-</td>
<td>-</td>
<td>50.0 ± 2.9 ab</td>
<td>15.8 ab</td>
<td>-</td>
</tr>
<tr>
<td>Sand, organic peat, perlite (1:1:1 v/v/v)</td>
<td>Freshwater</td>
<td>30.0 ± 1.7 a</td>
<td>7.9 a</td>
<td>62.2 ± 0.9 abc</td>
<td>9.9 ab</td>
<td>1.1 ± 0.3 b</td>
</tr>
<tr>
<td></td>
<td>Saltwater</td>
<td>-</td>
<td>-</td>
<td>55.6 ± 0.9 abc</td>
<td>14.0 ab</td>
<td>-</td>
</tr>
</tbody>
</table>

*Species without germination. A. halimus did not germinate under the greenhouse conditions tested. Values represent the mean ± standard error of the mean (SEM) of at least three experiments performed in triplicate (n = 30). In the same column, values followed by different letters are significantly different at p < 0.05 (Tukey HSD test).*
PCA analysis revealed that overall, salinity negatively impacted germination rates and delayed MGT across the species (Figure 1), which aligns with previous studies on other halophytes, such as Cakile maritima, C. quinoa, and different Salicornia and Sarcocornia ecotypes [37,47,48]. For most halophytes, germination and seedling emergence are strongly inhibited at salt concentrations much lower than those endured by adult plants, since at the adult stage, plants develop morphological, physiological, and biochemical adaptations to salt stress in the whole plant or tissues [10]. In the wild, saline conditions can inhibit the germination of halophytic species, leading to an excessive production of reactive oxygen (ROS) and nitrogen (RNS) species [49,50]. Under normal production, ROS/RNS react as secondary messengers in a variety of cellular processes, but their excessive production in saline conditions, for example, results in oxidative damage to lipids, proteins, and DNA, ultimately resulting in cell death [51]. To regulate the excessive production of ROS/RNS, plant cells rely on efficient enzymic and nonenzymic antioxidant mechanisms [52]. Therefore, seed germination in hostile environments, including saline settings, relies on the efficiency of the antioxidant defense system and varies with species and type of stress [53]. However, seeds can remain viable for long periods and germinate when conditions are favorable, typically in early spring when rain reduces soil salinity and temperatures rise. In contrast, glycophytic seeds only survive for short periods under high salinity [45].

The PCA biplot indicates a differential response to substrates and salinity levels, with coco peat and vermiculite mixes generally showing positive correlations with higher germination rates (Figure 1). Coco peat is made from shredded coconut husks and combines the water retention of vermiculite with the air retention of perlite [54]. Considering that the most essential step during germination is the uptake of water (imbibition) by the seeds, coco peat would allow the correct hydration required for embryo growth and endosperm rupture [55].

Figure 1. Principal components analysis (PCA) biplot of the germination of halophytes cultivated in different conditions of irrigation salinity and substrate. The graph represents the loadings on PC1 and PC2 of the parameters and the data scores of the samples. The red letters correspond to the studied species. MC: M. crystallinum; SR: S. ramosissima; MM: M. marina; AA: A. Arenaria; PO: P. oleracea. The blue letters correspond to the substrate followed by the irrigation water (substrate-irrigation water). Substrates: P: perlite; V: vermiculite; CP: coco peat; S: sand; CP + P: coco peat + perlite (1:1 v/v); CP + V: coco peat + vermiculite (1:1 v/v); P + V: perlite + vermiculite (1:1 v/v); S + OP + P: sand, organic peat + perlite (1:1:1 v/v/v). Irrigation water: F: freshwater; S: saltwater.
Seed treatments were applied to *M. marina* and *A. arenaria*, both with GRs lower than 50% (Table 2). Scarification with sulfuric acid increased the GR of *M. marina* by 42.2% (one-way ANOVA, Table S4 of the Supplementary Materials). In a previous study on the germination of *M. marina* under controlled conditions and freshwater irrigation, the highest GR was obtained after mechanical scarification (98%), followed by freezing at −20 °C (93%) and heating (25%), compared with the control (5%) [28]. Seeds from *M. marina* exhibit a strong dormancy due to the presence of a hard seed coat that prevents germination [28]. This coat-imposed dormancy, known as hardseededness, prevents seed water uptake, so that germination and the development of young seedlings occurs when evaporation and soil salinity decrease after the first rains [56,57]. Chemical scarification by sulfuric acid damages the surface of the seed coat, which increases the absorption of water and oxygen by the seeds [58,59], therefore improving germination. Scarification with ultrasounds was beneficial for *A. arenaria* (35.5% improvement). The use of ultrasounds is a simple, quick, and non-destructive method to break seed dormancy, causing fissures in the protective coating surrounding the pericarp (seed coat) and seed, increasing seedling moisture [60]. This system has been applied to break dormancy of seeds from different species, such as those from *Atriplex lentiformis* (Torr.) S. Watson, *Cuminum cyminum* L., *Zygophyllum eurypterum* Boiss. & Buhse, *Hordeum* spp (barley), *Thymus vulgaris* L. (thymes) and *Helianthus annuus* L. (sunflower) seeds [61–64].

**Table 2.** Germination rates (GRs, %) and improvement rates (IRs, %) in relation to the control group (without treatment) of seeds of *M. marina* and *A. arenaria* after the application of germination improvement treatments.

<table>
<thead>
<tr>
<th>Type of Treatment</th>
<th>Specific Treatment</th>
<th><em>M. marina</em></th>
<th><em>A. arenaria</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>GR</td>
<td>RI</td>
</tr>
<tr>
<td>Chemical treatment</td>
<td>Gibberellic acid 1 g/L, 24 h</td>
<td>35.0 ± 1.2 a</td>
<td>+23.9</td>
</tr>
<tr>
<td>Chemical scarification</td>
<td>Sulfuric acid 50%, 10 min</td>
<td>53.3 ± 1.7 ab +42.2</td>
<td>26.7 ± 1.3 abcd</td>
</tr>
<tr>
<td>Mechanical scarification</td>
<td>Ultrasounds, 15 min</td>
<td>25.0 ± 1.2 a</td>
<td>+13.9</td>
</tr>
<tr>
<td>Soaking</td>
<td>Water at RT, 24 h</td>
<td>23.3 ± 0.7 a</td>
<td>+12.2</td>
</tr>
<tr>
<td></td>
<td>W60</td>
<td>36.7 ± 1.3 a</td>
<td>+25.6</td>
</tr>
<tr>
<td></td>
<td>W75</td>
<td>30.0 ± 1.0 a</td>
<td>+18.9</td>
</tr>
<tr>
<td></td>
<td>W75W-4</td>
<td>38.3 ± 0.7 a</td>
<td>+27.2</td>
</tr>
<tr>
<td></td>
<td>W-4W75</td>
<td>40.0 ± 1.7 a</td>
<td>+28.9</td>
</tr>
<tr>
<td></td>
<td>W-4</td>
<td>26.7 ± 1.2 a</td>
<td>+15.6</td>
</tr>
<tr>
<td>Thermal shock</td>
<td>W60</td>
<td>35.0 ± 1.2 a</td>
<td>+23.9</td>
</tr>
<tr>
<td></td>
<td>W75</td>
<td>53.3 ± 1.7 ab +42.2</td>
<td>26.7 ± 1.3 abcd</td>
</tr>
<tr>
<td></td>
<td>W75W-4</td>
<td>25.0 ± 1.2 a</td>
<td>+13.9</td>
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<tr>
<td></td>
<td>W-4W75</td>
<td>23.3 ± 0.7 a</td>
<td>+12.2</td>
</tr>
<tr>
<td></td>
<td>W-4</td>
<td>36.7 ± 1.3 a</td>
<td>+25.6</td>
</tr>
<tr>
<td></td>
<td>W75</td>
<td>30.0 ± 1.0 a</td>
<td>+18.9</td>
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<tr>
<td></td>
<td>W-4</td>
<td>26.7 ± 1.2 a</td>
<td>+15.6</td>
</tr>
</tbody>
</table>

Values represent the mean ± standard error of the mean (SEM) of at least three experiments, each performed in triplicate (n = 30). In the same column, GR values followed by different letters are significantly different at p < 0.05 (Tukey HSD test).

Table 3 presents a summary of the best germination conditions, including the specific treatment to improve germination if applicable, for each halophyte studied. It is important to note that achieving an optimized germination rate does not necessarily guarantee the successful growth and development of seedlings, especially under saline conditions [65]. Therefore, further optimization studies are required for subsequent cultivation phases to ensure the viability and healthy development of the plants.

**Table 3.** Best germination conditions for the studied halophyte species.

<table>
<thead>
<tr>
<th>Species</th>
<th>Irrigation</th>
<th>Substrate</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Mesembryanthemum crystallinum</em> L.</td>
<td>Freshwater</td>
<td>Coco peat, vermiculite (1:1 v/v)</td>
<td>n.a.</td>
</tr>
<tr>
<td><em>Salicornia ramosissima</em> J. Woods</td>
<td>Freshwater</td>
<td>Coco peat, vermiculite (1:1 v/v)</td>
<td>n.a.</td>
</tr>
<tr>
<td><em>Medicago marina</em> L.</td>
<td>Freshwater</td>
<td>Vermiculite</td>
<td>Sulfuric acid 50%, 10 min</td>
</tr>
<tr>
<td><em>Ammophila arenaria</em> (L.) Link</td>
<td>Freshwater</td>
<td>Coco peat</td>
<td>Ultrasounds, 15 min</td>
</tr>
<tr>
<td><em>Portulaca oleracea</em> L.</td>
<td>Freshwater</td>
<td>Coco peat</td>
<td>n.a.</td>
</tr>
<tr>
<td><em>Atriplex halimus</em> L.</td>
<td>No germination</td>
<td>No germination</td>
<td>No improvement</td>
</tr>
</tbody>
</table>

n.a. Not applicable.
4. Conclusions

This study underscores the importance of optimizing germination conditions for halophytes to ensure successful saline agriculture under greenhouse conditions, making it feasible for large commercial-scale crop production. Freshwater irrigation and specific substrates, particularly coco peat combined with vermiculite for *M. crystallinum* (GR, 52.2%) and *S. ramosissima* (GR, 66.7%), significantly enhanced germination rates. Seed treatments, such as scarification with sulfuric acid for *M. marina* (GR, 53.3%) and ultrasounds for *A. arenaria* (GR, 53.3%), can further improve germination for species with low initial rates. The results obtained from this work provide a preliminary foundation for optimizing the early stages of the cultivation process. Ongoing cultivation trials are being conducted to refine the saline cultivation methods for selected species identified in this study. These trials aim to enhance biomass production and improve the biochemical profiles of the plants.

**Supplementary Materials:** The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/horticulturae10080872/s1, Table S1: Traditional uses, and nutritional and functional properties of *Mesembryanthemum crystallinum* L., *Salicornia ramosissima* J. Woods, *Medicago marina* L., *Ammophila arenaria* (L.) Link, *Portulaca oleracea* L., and *Atriplex halimus* L.; Table S2: Two-way ANOVA of effects of irrigation, salinity, and their interactions on MGT in F-values at 0.05 level; Table S3: Two-way ANOVA of effects of irrigation, salinity, and their interactions on seed MGT in F-values at 0.05 level; Table S4: One-way ANOVA of effects of treatment to improve seed germination in F-values at 0.05 level. References [9,66–80] are cited in Supplementary Materials file.

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**Data Availability Statement:** The original contributions presented in the study are included in the article/Supplementary Materials, further inquiries can be directed to the corresponding author.

**Conflicts of Interest:** The authors declare no conflicts of interest.

**References**


22. Rodrigues, M.J.; Monteiro, I.; Castaño-de Loaiza, V.; Placines, C.; Oliveira, M.C.; Reis, C.; Caperta, A.D.; Soares, F.; Pousão-Ferreira, P.; Pereira, C. Growth performance, in vitro antioxidant properties and chemical composition of the halophyte Limonium algarvense Erben are strongly influenced by the irrigation salinity. Ind. Crops Prod. 2020, 143, 111930. [CrossRef]


34. Osman, A.E.; Ghassali, F. Effects of storage conditions and presence of fruiting bracts on the germination of Atriplex halimus and Salsola vermiculata. Exp. Agric. 1997, 33, 149–155. [CrossRef]
43. Mikhail, G.S.; Meyer, S.E.; Pendleton, R.L. Variation in germination response to non-invasive species and salinity in shrub Atriplex species. J. Arid Environ. 1992, 22, 39–49. [CrossRef]
45. Gul, B.; Ansari, R.; Flowers, T.J.; Khan, M.A. Germination strategies of halophyte seeds under salinity. Environ. Exp. Bot. 2013, 92, 4–18. [CrossRef]
52. Hasanuzzaman, M.; Bhuyan, M.H.M.B.; Anee, T.I.; Parvin, K.; Nahar, K.; Mahmud, J.A.; Fujita, M. Regulation of ascorbate-glutathione pathway in mitigating oxidative damage in plants under abiotic stress. Antioxidants 2019, 8, 384. [CrossRef]


77. Nemzer, B.; Al-Taheer, F.; Abshiru, N. Phytochemical composition and nutritional value of different plant parts in two cultivated and wild purslane (Portulaca oleracea L.) genotypes. Food Chem. 2020, 320, 126621. [CrossRef]

