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Threats of abiotic factors to the germination of Salicornia europaea L. and the measures for wetland species conservation

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Abstract

Salicornia europaea L. is a salt-tolerant wetland plant with great economic value, but the population of *S. europaea* has been drastically reduced because its survival environment is seriously threatened. In this study, from the perspective of the germination stage of *S. europaea*, the relationship between the germination of *S. europaea* and different abiotic factors was investigated, the possible causes of environmental changes leading to the degradation of this species in coastal wetlands was explored, and the direction of wetland restoration, and optimized the conditions for artificial germination of *S. europaea* seeds was proposed. The results showed that *S. europaea* was sensitive to changes of GA_3 concentration, salinity and temperature during the germination stage, the effect of water immersion treatment on seed germination was concentrated in the late stage of germination, and high salinity, low temperature and prolonged submergence of the seeds all led to limited seed germination. Meanwhile, the combination of analytic hierarchy process and orthogonal experiments were used to screen the range of these factors and the combination of optimal germination conditions, The optimal combined germination conditions was identified as 2000 mg/L gibberellin, 5‰ culture salinity, 20 ℃ culture temperature and 12-hour soaking time. and was validated having practical significance. In conclusion, the results of this study provide an insight to the relationship between degradation of wetland plant populations and abiotic factors, and help in the conservation of plant diversity and the domestication and utilization of wild wetland plants.

Introduction

Salicornia europaea L. (*S. europaea*) is a succulent annual plant in the family of Chenopodiaceae, it is characterized by fleshy segments leaves and terminal inflorescences in the form of spikes (Scott 1977). *Salicornia* a genus with 64 species(Cárdenas-Pérez et al. 2021) , is globally distributed in various types of coastal wetlands, pans and lakes (Piernik et al. 2015). However, according to Flora Reipublicae Popularis Sinicae (FRPS, 1979) and Flora of China (FOC, 2003), *S. europaea* is the only naturally occurring species of the genus *Salicornia* in China. Its distribution characteristics are closely related to soil salinity. *S. europaea,* is renowned for its extremely high salt tolerance, with the mechanisms such as osmotic adjustment through high water storage and dilution, substantial sodium accumulation primarily in its branches (Ushakova et al. 2005), even in the absence of salt glands and salt capsules(Grigore &Toma 2020). The up-regulation of Na transporter genes *NHX1* and *HKT1* under salt stress plays an important role in transporting and isolating Na(Lv et al. 2012). Consequently, *S. europaea* can thrive in environments with salt concentration exceeding 1000 mM NaCl (Lv et al. 2012)., unlike its congeners *Salicornia herbacea L.* and *Salicornia bigelovii Torr*. *S. europaea* exhibits a preference for cooler climate. Thus, it predominantly inhabits coastal wetlands, Salt Lake marshes, and arid saline-alkali lands of mid-high latitude regions.

S. europaea possesses significant economic value, particularly in the realms of food production and habitat restoration. Amidst soil salinization and the crisis of freshwater resources, saline plants such as *S. europaea* can be cultivated in saline soils as an alternative food crop to help mitigate the food

shortages caused by population growth and global warming(Singh et al. 2014). Meanwhile, *S. europaea* is excessively employed in environmental remediation, showing potential in emerging pharmaceutical pollutant uptake (Barreales-Suárez et al. 2021), as hyper-accumulators of heavy metals such as zinc, lead, nickel, chromium, etc., in contaminated soils and water bodies (Khanlarian et al. 2020), and in nitrogen fixation capacity and enrichment effect of diazotrophic bacteria in saline soils(Hrynkiewicz et al. 2019). It is also commonly used as a biological filter for treating dissolved inorganic nitrogen and inorganic phosphorus in maricultural wastewater and eutrophic seawater(Webb et al. 2012). Consequently, *S. europaea* as a salt-tolerant plant species, warrants attention and conservation due to its great potential in seawater purification.

The habitat of *S. europaea* is under increasing pressure, leading to its habitat gradually deteriorated. According to the survey, *S europaea* in Hokkaido area of Japan has been designated as a protected species by the Japanese government as a natural relic. In the salt lake wetlands of the Mongolian Plateau, *S. europaea* is sparsely distributed, facing a severe decline in population size and a high risk of extinction. The *S. europaea* community in the wetland at the mouth of the Wei River in Laizhou Bay, disappeared in the last two years. The reduction of *S. europaea* populations due to abiotic factors is a cause of concern. Human activities cause climate change (Petrescu et al. 2015) and land change (Xindi et al. 2020), which have a huge impact on the degradation of wetland ecosystem . Wetland plants are extremely sensitive to climate change(Capon et al. 2013), and extreme temperature variation directly harm wetland functions, altering hydrological dynamic of wetlands, causing uneven soil water retention (Aquino et al. 2021, Chen et al. 2014), resulting in spatial-temporal changes of soil salinity (Cantelon et al. 2022). The frequent occurrence of drought and flood causes wetland fragmentation (Price &Berkowitz 2020). These factors may subject the seeds of wetland plants to low-temperature stress, water stress, and salt stress during the germination stage.

According to surveys, the wetland area at the estuary of the major rivers in Laizhou Bay decreased year by year in the last 50 years , due to land reclamation and salt-field farming(Wang et al. 2023)Changyi Meteorological Station in Weifang City reported that the local temperature increased from 12.2°C to 13.2°C in the last 50 years, accompanied by an increase in the frequency of extreme temperature variations and extreme tidal outbursts, These environmental changes have led to retrogression and succession coastal wetland plants (Zhang et al. 2021). The rapid disappearance of the plant community due to the abiotic factors is weakening the ecological capacity of coastal wetlands.

In this study, our objectives are to investigate the relationship between the germination of *S. europaea* and different abiotic factors, explore the possible causes of environmental changes leading to the degradation of this species in coastal wetlands, and to determine the optimal conditions for artificial germination of *S. europaea*. Our specific goals include: 1) analyzing the influence of individual factors on seed germination. 2)assessing comprehensive seed growth under the combined influence of multiple factors. 3) identifying optimal conditions for germination and verifying their effectiveness. 4) providing conservation recommendations for *S. europaea*. This research will contribute valuable insight into understanding the causes of wetland ecological degradation, and help establish efficient models for the

artificial cultivation of wild *S. europaea*, facilitating the restoration and domestication of wild salt-tolerant plant population and supporting habitat restoration as a tool species for fishery and biodiversity conservation.

Materials and Methods

Study area and Materials

Field-bearing seeds of *S. europaea* were collected from a natural wetland at the mouth of the Wei River in Laizhou bay, Changyi City, Shandong Province (119°29'E, 37°4'N) in Oct.2022. These seeds were used as experimental materials (Fig. 1). After harvesting, the seeds underwent a thorough screening process, eliminating immature or damaged seeds. Subsequently, they were, quickly rinsed with distilled water to remove impurities and surface moisture. The cleaned seeds were sealed and stored in a dry and light-proof, low-temperature refrigerator.

Effect of single-factor treatments on the germination of S. europaea

In March 2023, germination experiments were carried out in the light-temperature incubator. The study involved artificial control factors and environmental change factors. Gibberellin GA_3 (GA_3), a plant hormone, was used as an artificial adjustment condition to enhance of germination environment. The environmental factors including salinity of soaking and irrigation water (Culture Salinity), temperature and soaking pretreatment time (Soaking Time) as was used to stimulate *S. europaea* seeds. The germination and development of seeds in each group were observed to investigate the influence of single factors on seed germination.

Germination was conducted using the Petri dish paper method. Prior to the experiment, seeds with full and hard shape were selected as germination objects. The seeds' shells were sterilized by soaking them in anhydrous ethanol for 12mins. Two layers of filter paper were placed in the sterilized and dried petri dishes as the bottom base, and 50 pre-treated seeds were evenly spread on them. Each group had 3 replicates. Each petri dish was sprayed with equal amount of irrigation water every day, maintaining incubator humidity between 50%-90%. The numbers of germinated seeds were recorded daily, the germination was defined as the development of embryonic root to the equal length, Seeds failed to germinate in 7d were marked as non-germination.

Single-factor condition was set as follows: 1) GA_3 treatment conditions: 0 mg/L, 100 mg/L, 500 mg/L, 1000 mg/L, 1500 mg/L, 2000 mg/L, 2500 mg/L GA_3 mixed with salinity of 2.5‰ (about 42.74 mM NaCl). 2) Culture salinity treatment conditions: culture salinity of 0‰, 2.5‰, 5‰ (about 85.47 mM NaCl), 10‰ (about 170.94 mM NaCl), 15‰ (about 256.41 mM NaCl), 20‰ (about 341.88 mM NaCl) and 25‰ (about 427.35 mM NaCl), with a soaking time of 24 h and culture temperature of 25°C. 3) Culture temperature treatment conditions : culture temperatures of 5°C, 10°C, 15°C, 20°C, and 25°C for solid-temperature conditions, as well as alternating temperature regimes of 16h for 20°C/8h for 10°C and 16h for 25/8h for 15°C variable temperature conditions., with a culture salinity of 2.5‰ and soaking time of

24 h. 4) Soaking time treatment conditions: soaking for 6h, 12h, 24h, 36h and 48h, with a culture salinity of 2.5‰, and a temperature of 25°C.

Effect of multi-factor treatments on S. europaea germination and the screening of optimal germination conditions

The germination effects under multifactorial treatments were analyzed and optimal germination conditions screened by combining the analytic hierarchy process (AHP)(Veisi et al. 2022) and the orthogonal experimental method (Ranil et al. 2015).

A more comprehensive selection of levels was achieved by integrating various germination indices beside germination percentage. The AHP were employed to normalize the indexes of each factor and assign weights to the scores. The AHP method comprised the following steps: establishing a hierarchical structure and setting quantitative values (Table 1), constructing a judgment matrix and inviting 10 experts to score the relative importance of growth indices through pairwise comparison according to the quantitative values (Table 2), Calculating the weight values ω of each factor, with a consistency test of the judgment matrix (CR = 0.05 < 0.1), indicating that no conflict in the scoring results., Evaluating the results of the single-factor experiment based on ω, and comprehensively scoring and ranking the plant germination and growth. Selecting the top 3 levels with the highest comprehensive weight score K_i for each factor for a four-factor three-level orthogonal experiment. The seed disinfection and sowing methods of the orthogonal experiment were with the same as *2.2*, involving 9 experimental groups and 9 control groups.

Measurement and Analysis

At the end of the germination stage, 15 seed samples were randomly selected from each petri dish, and the main root length and stem length of the germinated seeds were measured using a ruler (minimum scale 1mm) and summed up to obtain the total length(*TL*/cm). The seed weight (*Wt*/mg) was measured using a precise balance (minimum scale 0.1mg), and the mean value was calculated. Additionally, the following seed indices were recorded: germination percentage (*GP*/%), Germination Index（*GI*）and vigor index:

Germination Percentage (*GP*) = $\frac{\mathrm{Finalnumberofseedlingsemerged}}{\mathrm{Totalnumberofseeds}} \times 100$(Ozel et al. 2021)

Germination Index (*Gi*) = $\sum \frac{Gt}{Dt}$ (*Gt*: number of germination on day t. *Dt*: corresponding germination day) (Gour et al. 2023)

Vigor Index (*Vi*) = $G_i \times S$ (*S*: growth potential, represented by root length)(Deng et al. 2014)

Comprehensive weight score (K_i) = $\sum G_n \cdot \omega_n$ (*G*: index value, ω: weight values. *n*: time)

Optimal germination combination (T_w): Selection of the combination of conditions with the highest K_i using range analysis and analysis of variance measurement data was recorded using Excel 2016, growth indicators was calculated and hierarchical analysis was performed; IBM SPSS Statistics 27 was used for range, variance and correlation analysis. Graphical figures were created using Origin 2023.

Result and Analysis

Effect of single factors on seed germination percentage and germination speed.

Introduction of GA_3 effectively enhanced the germination percentage of *S. europaea* seeds (Fig. 2a). All GA_3 treatment groups exhibit significantly higher germination vigor than the group without GA_3 addition. Notably, the group treated with 2000mg/L GA_3 demonstrated the highest germination percentage (88.67%). As GA_3 concentration increases, the total germination percentage of seeds also increased, but, GA_3 showed inhibitory effect on seed germination when the concentration was up to 2500mg/L. Seeds treated with GA_3 concentrations higher than 500mg/L germinate quickly, primarily within 2–3 days, displaying a more stable and concentrated germination pattern. In contrast, the 0mg/L, 100mg/L and 500mg/L groups showed a less pronounced and extended germination period (Fig. 2b). Seed in these groups reached a germination percentage exceeded 50% by the second day, and ceased germinating after the fourth day.

In terms of salinity stress, the germination percentage declined with the increase of salinity level (Fig. 3a). Salinity levels above 10‰ significantly reduced the germination percentage to lower than 40%, with only 13.33% when the salinity was 25‰. Salt stress also significantly affected the germination speed and germination index of *S. europaea* (Fig. 3b). the seeds exposed to 2.5‰ and 5‰ salinity level exhibited a germination percentage of around 65%., but their germination index was below 10. Despite *S. europaea*'s, extremely high salt tolerance, it was highly sensitive to high salinity during germination.

Regarding temperature, the optimal germination temperature range for *S. europaea* is concentrated between 20℃to 25℃ (Fig. 4a). Treatment groups with temperature difference simulating day-night variation 10/20℃ and 15/25℃exhibited higher germination percentages than constant temperature treatment groups, with 15/25℃ showing the highest germination percentage (82.67%), which was also similar to the natural environmental conditions of *S. europaea* germination. Germination percentage was below 30% in the treatment conditions below 10℃, nearly reaching zero at 5℃. The low temperature of 10℃ also delayed the germination of seeds, with only a few seeds germinating during the first 5days, following by more intensive germination starting on the 6th day., However the germination index remained low. The germination indices under variable temperature condition were higher than those treated with constant temperature. The low temperature treatments of 5℃ and 10℃, exhibited the lowest germination indices, there was no significant difference in germination indices among other groups (Fig. 4b).

The duration of soaking didn't significantly affect the germination percentage of *S. europaea* seeds(Fig. 5a)The germination percentage change trend was relatively dense. Meanwhile, the difference in germination index was either not significant (Fig. 5b). The seeds soaked for 6h exhibited a slightly lower germination speed than those of other groups.

The effect of single factor on seed growth

GA_3 continued to influence the seed growth during the germination stage (Fig. 6a). Seeds treated with 100mg/L, 500mg/L and 2000mg/L exhibited better root development, while those treated with 1500mg/L and 2000mg/L showed the best stem growth. Conversely, the seeds treated with 0mg/L and 2500mg/L had the shortest root length. Although there was a significant difference in the *Wt* of *S. europaea* among groups, the fluctuation of the difference was minimal, suggesting that GA_3 has a limited effect on the *Wt* of seeds after germination. Considering the root length, stem length and *Wt* indicators, the best plant growth occurred under treatment of 1500mg/L and 2000mg/L GA_3.was

High-salinity germination environments significantly limited the later-stage development of *S. europaea* (Fig. 6b). Low-salt conditions (2.5‰) reduced the germination rate but had no significant impact on growth. However, groups exposed to salinity levels above 5‰ experienced a significant reduction in total length and weight, with roots affected more than stems and weight.

Temperature had a significant influence on seed germination and growth (Fig. 6c). The best root and stem growth occurred under variable temperature conditions of 25/15℃. Temperature below 15℃ significantly affected the root and stem development of *S. europaea*. Seeds subjected to 5℃ and 10℃ exhibited minimal germination, and those germinated showed slow development, with insufficient data complete the analysis of *TL* indicators. The *Wt* of seeds in these two treatments was significantly lower than that of other groups.

As shown in Fig. 4d, 12 hours and 36 hours of soaking resulted in greater root growth compared to other soaking time. Stem length of groups was highest in groups with soaking time of 12h, 24h and 36h (Fig. 6d). The group with soaking time of 12h had the largest average *Wt* while the group soaked for 36h had the smallest.

Optimal germination conditions and influence intensity of each factor.

Based on the Analytic Hierarchy Process (AHP) and orthogonal experimental method, the influence of each factor on seed germination was evaluated according to the indicator results of the single factor experiment in 2.2, and the comprehensive weight scores K_i were calculated (Table 3). The influence intensity of each factor on seeds within this range was determined to be: GA_3 concentration > culture temperature > culture salinity > soaking time. According to the K_{i-max} of each column, the optimal germination condition T_w under the predetermined factor conditions was identified as a combination of 2000mg/L gibberellin concentration, 5‰ culture salinity, 20℃ culture temperature and 12-hour soaking time, which performed better than other combinations in practice.

A verification experiment was conducted on this optimal condition(*Tw*)(Table 4). The results showed significant advantage in Germination index (*GI*) and Vigor index (*VI*) comparted to other condition combinations, further supporting its efficacy. T *GI* and *VI* in the T_{wi} condition had significant advantages, compared with the *VI* and K_i under the condition combination of 2000mg/L GA_3 concentration and 5‰ culture salinity and 25℃ culture temperature and 48h soaking time, there was no significant advantage, but compared with the *VI* and K_i of other condition combinations, there was significant advantage, *TL* had no significant advantage.

The general linear model (GLM) univariate analysis was performed on the orthogonal experiment results (Table 5). The results showed that GA_3 and culture temperature had extremely significant effects on K_i, culture salinity had significant effects, and soaking time had no significant effects. According to the *F* value, the effects of each factor on K_i within this level range were: GA_3 concentration > culture temperature > culture salinity > soaking time, which was consistent with the range analysis results. The Student-Newman-Keuls (S-N-K) test suggested that the T_w under the given conditions was: 2000mg/L GA_3 concentration and 5‰ culture salinity and 20℃ culture temperature and 12h soaking time, which was also consistent with the range analysis results.

Correlation analysis of abiotic factors and growth indicators

Correlation analysis of *GP*, *TL*, *Wt*, *Gi* and *Vi* with 4 abiotic factors was conducted (Fig. 7). *TL* exhibited significantly positive correlation with culture temperature, *Gi, Wt* and *Vi* ($P < 0.05$). *Wt* showed significant positive correlation with culture temperature, GA_3 concentration, *Gi* and *Vi* ($P < 0.05$). *Gi* exhibited significant positive correlation with culture temperature, GA_3 concentration and *Vi* ($P < 0.05$). *Vi* was significantly positively correlated with culture temperature and GA_3 concentration ($P < 0.05$). Culture temperature showed significant positive correlation with culture salinity ($P < 0.05$), while *GP* was significant positive correlated with culture salinity.

Discussion

GA_3 significantly regulates the germination of S. europaea

Plants under environmental stress have limited capacity to produce their own gibberellins. the introduction of exogenous gibberellins (GA3) by applying has been effectively verified to induce plant germination in many plant experiments (El Hamdaoui et al. 2021, Xiong et al. 2021), This study further confirms the positive impact of GA3 on *S. europaea* germination and growth during the early stages. 1×10^{-3} M (about 346 mg/L) of exogenous GA_3 treatment can alleviate the salt stress of 5% NaCl concentration and improve the germination percentage of seeds(Ungar 1977), Our study shows that the plant hormone GA_3 is widely involved in the growth of *S. europaea* at the germination stage, causing differences in indicators such as germination percentage, germination speed, plant weight, stem length and length ratio. Within the range of 100mg/L to 2000mg/L, this effect is positive, but showed inhibit

tendency when the concentration of GA_3 was up to *S.* 2500mg/L. The effect of higher GA3 concentration treatment and other plant hormone on the growth of *S. europaea* during the germination needs further exploration.

Salt-tolerant plants are not salt-tolerant at the germination stage

S. europaea is known for its high salt tolerance, which allows it to adapt to various ecological environments. However, this tolerance is not consistent across all stages of its life cycle, particularly during germination. Ungar reported (1977)that *S. europaea* seeds can germinate under salinity between 10‰ and 50‰ but significantly low than those, under salinity below 10‰ and the distilled water. The higher the NaCl concentration showed the more obvious inhibition on seed germination (Orlovsky et al. 2016). Also as observed in our study, the germination rate declines sharply with salinity increases, indicating that the seeds are less tolerant to salt during this stage. In addition to reduced germination, high salinity also negatively impacts growth indicators such as stem length, root length, and weight.

Previous research have shown that *S. europaea* can thrive with increased weight under low NaCl concentration of 85mM (4.2‰) but experience a gradual decline in weight at higher salinity(Moghaieb et al. 2004), In this study, we observed that the highest weight and length were achieved under the 2.5‰ salinity treatment. This discrepancy may be attributed to variations in culture conditions and inherent seed characteristics. It has been reported that Soil salinity can increase due to factors such as tidal action, engineering construction, and wastewater discharge, which can affect coastal wetlands (Li et al. 2014). Changes in salinity levels have a direct impact on plant diversity in these ecosystems, potentially leading to a decrease in the population of S. europaea (Hou et al. 2020). Therefore, as our result showed, while *S. europaea* exhibits high salt tolerance in later stages of development, it is notably sensitive to high salinity during germination. The groundwater level of the coastal wetlands in the southern part of Laizhou Bay shows a periodic decrease during the dry season (April). Since 1976, the extent of seawater intrusion into coastal wetlands has increased year by year (Zhang et al. 2021), resulting in drastic changes in soil salinity in the region's wetlands. The replacement of typical plant niches by mesophytes and xerophytes within the community is the direct reason for the decrease in plant diversity level in coastal wetlands and the decrease in the population of *Salicornia Europaea L.*.

The response of the seed to temperature and water stress varies with different ecological environments and its own characteristics

S. europaea demonstrates some degree of low-temperature tolerance and can germinate at temperature as low as 10°C and even lower, but the germination percentage and germination time of the seeds were seriously affected. Unfavorable germination temperature can disrupt the physiological and biochemical processes of the seeds(Monajjem et al. 2023), Each plant species has an optimal germination temperature range, and for *Salicornia*, this range falls between 20°C to 35°C(Ajmal Khan et al. 2000, Ungar 1977). Our study confirmed that *Salicornia europaea L.* germinates most effectively at temperatures between 20°C to 25°C. Furthermore, variable temperature conditions, which mimic natural

day-night temperature fluctuations, had a slight positive effect on the germination rate and length of seeds compared to constant temperature conditions in single factor experiments, although the germination speed and weight were slightly lower.

Related research has shown that water stress represses seed germination by modulating glycometabolism, anaerobic respiration and phytohormone biosynthesis -related cascades (Zhou et al. 2021). Although short-term soaking has minimal effects on the germination rate and germination time of *S. europaea*, it had a more significant impact on growth indicators. This difference may be attributed of the fact that seed metabolism is minimal during germination, mainly relying primarily on the stored sugar for energy (Zhou et al. 2018). In contrast, the post-germination phase is highly sensitive to the environment conditions, and the effects of soaking treatment may become apparent during this stage. Subsequent studies are needed to investigate longer soaking time and post germination submergence to simulate the impact of seawater backflow, floods and long-term flooding wetlands on seed germination. This may shed light on the reasons behind the decline in the population of *S. europaea* in the estuarine area in north China over the decade.

Artificial cultivation patterns and recommendations for species conservation

Certain studies have suggested that mild stress environment induced by abiotic factors can serve as trigger signals for seeds, promoting seed germination and seedling growth (Noman et al. 2018, Srivastava et al. 2021). Our experiments revealed that the optimal germination conditions are not a simple combination of individual K_{i-max} conditions from singe factor experiment. By comparing the results of orthogonal experiment combination with the verification experiment, we identified the theoretically optimal germination conditions performed better in practice. multiple factors act together, the environmental conditions with high gibberellin concentration, low salinity, low temperature and short soaking time are more conducive to the germination and growth of *S. europaea* seeds. While artificial regulation can improve the germination efficiency of *S. europaea* seeds by adjusting environmental factors, the degree of adjust should be carefully considered. The correlation heatmap suggested that, within the selected condition range, most abiotic factors have a positive effect on germination rate and other growth indicators. However, salinity exhibited a negative trend on germination rate, even if this effect did not reach statistical significance ($P < 0.05$). Therefore, when cultivating *S. europaea* in controlled environments, seeds should be soaked with exogenous hormones in advance to release germination signals, and it is essential to focus on the humidity and salinity of the soil and irrigation water. Additionally, human interventions aim to avoid abrupt environmental changes during the cold season, and customize combinations of abiotic conditions for different cultivation purposes.

To conserve the *S. europaea* populations in the wild, we recommend the following:

Construct riverbanks to protect ecologically and economically valuable waterfront wetlands, to minimize the risk of inundation caused by hydrological changes.

Expand the scope of wetland ecological protection areas, while controlling the proximity of seawater aquaculture salt fields outside the protection area, to avoid pumping groundwater or discharging aquaculture tail water causing an increase of soil salinity in wetland.

When establishing artificial cultivation conditions, pay close attention to the impact of salinity on seed germination during soaking treatment and irrigation.

Continue to investigate the causes of abiotic conditions affecting the germination effect of *S. europaea*, providing guidance for the improvement of wetland environment.

Explore the possibility of conducting artificially field seeding of *S. europaea*, either in the native habitat of *S. europaea* after condition improvement or in an *ex-situ* natural wetland with robust ecological vitality.

Conclusion

The results of this study highlighting several key findings: High salinity above 10‰ has a significant inhibitory effect on the germination and growth of *S. europaea*, despite the plants high salt tolerance in later growth stages. the total length and weight decrease with the increase of salinity; Germination of *S. europaea* is most successful at temperature range between 20°C and 25°C, and variable temperature conditions closer to natural day-night fluctuations can enhance germination; Short-term soaking treatments have minimal impact on germination rates but can affect post-germination growth indicators, suggesting a potential link to the decline of S. europaea populations in wetland ecosystems. The optimal germination conditions involve a combination of factors, including high gibberellin concentration, low salinity, low temperature, and short soaking times.

Artificial regulation can enhance germination efficiency by applying controlled environmental stress, but salinity levels during soaking treatments and irrigation should be carefully managed.

Conservation efforts for S. europaea in the wild should focus on protecting wetland ecosystems, minimizing saltwater intrusion.

Declarations

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Author contribution All authors contributed to the study conception and design. Zijun Luo: Conceptualization, Investigation, Formal analysis, Investigation, Data curation, Writing – original draft, Visualization. Wenjie Tian: Investigation, Writing & editing, Saisai Chi: Writing & editing, Data curation. Bo Sun: Investigation, Formal analysis. Jufa Chen: Conceptualization, Project administration. Qiufen Li:

Conceptualization, Investigation, Writing – review & editing, Supervision, Project administration, Funding acquisition, Resources.

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Tables

Table 1 Quantizer and meanings of AHP

Factor **i** versus factor **j**	quantizer
i is equally important as **j**	1
i is slightly more important than **j**	3
i is moderately more important than **j**	5
i is strongly more important than **j**	7
i is extremely more important than **j**	9
Intermediate values between adjacent scores	2、4、6、8

Table 2 Judgment layer matrix and weight values ω

index	*GP*	*Total Length*	*Gi*	*Vi*	ω
GP	1	6	8	4	0.63
Total Length	1/6	1	3	1/5	0.20
Gi	1/8	1/3	1	1/7	0.05
Vi	1/4	5	7	1	0.12
Amount	1.49	6.70	17.00	9.33	

Judgment layer matrix CR=0.05<0.01.

Table 3 Single factor K_i score

Source	Factor A		Factor B		Factor C		Factor D	
	GA_3 (mg/L)	K_i	Culture Salinity (‰)	K_i	Culture Temperature (℃)	K_i	Soaking time (h)	K_i
1	2000	7.14	0	3.64	25/15	4.28	12	4.47
2	1500	6.77	2.5	3.32	20	3.64	36	3.99
3	1000	5.70	5	2.22	20/10	3.46	24	3.95
4	2500	5.64	10	2.04	25	3.41	48	1.62
5	500	5.31	15	1.24	15	1.89	6	1.47
6	100	4.59	20	0.98	10	0.25	-	-
7	0	3.69	25	0.61	5	0.03	-	-

Table 4 Orthogonal experiment table L9(34) and experimental results analysis

	Factor A	Factor B	Factor C	Factor D	*GP*	*TL*	*GI*	*VI*	*Ki*
1	2000	0.0	20/10	12	78.00^{b}	1.68±0.15^{b}	19.87^{b}	11.52^{b}	6.23^{b}
2	2000	2.5	20	6	86.00^{b}	1.62±0.21^{bc}	16.92^{c}	11.00^{c}	5.95^{cd}
3	2000	5.0	25/15	48	82.00^{b}	1.85±0.07^{a}	21.23^{b}	15.07^{a}	7.09^{a}
4	1500	0.0	20	48	84.00^{b}	1.42±0.22^{e}	12.19^{d}	5.61^{e}	4.65^{g}
5	1500	2.5	25/15	12	86.00^{b}	1.57±0.13^{c}	20.68^{b}	10.96^{c}	6.03^{c}
6	1500	5.0	20/10	6	70.00^{c}	1.73±0.15^{b}	15.40^{cd}	8.62^{d}	5.71^{d}
7	1000	0.0	25/15	6	82.00^{b}	1.62±0.13^{bc}	16.09^{c}	8.53^{d}	5.58^{d}
8	1000	2.5	20/10	48	80.00^{b}	1.58±0.21^{bc}	14.18^{d}	8.22^{d}	5.36^{e}
9	1000	5.0	20	12	70.00^{c}	1.54±0.17^{d}	13.62^{d}	7.90^{d}	5.15^{f}
10	2000	0.0	20/10	12	84.00^{b}	1.64±0.11^{b}	22.45^{b}	13.92^{b}	6.60^{b}
11	2000	2.5	20	6	82.00^{b}	1.61±0.24^{bc}	17.67^{c}	10.60^{c}	5.89^{cd}
12	2000	5.0	25/15	48	88.00^{b}	1.77±0.15^{a}	22.18^{b}	15.75^{a}	7.09^{a}
13	1500	0.0	20	48	80.00^{b}	1.44±0.20^{e}	12.97	6.23^{e}	4.78^{g}
14	1500	2.5	25/15	12	80.00^{b}	1.58±0.09^{c}	21.98^{b}	10.55^{c}	6.03^{c}
15	1500	5.0	20/10	6	70.00^{c}	1.71±0.12^{b}	16.68^{cd}	8.67^{d}	5.74^{d}
16	1000	0.0	25/15	6	84.00^{b}	1.66±0.20^{bc}	18.70^{c}	9.35^{d}	5.91^{d}
17	1000	2.5	20/10	48	76.00^{b}	1.63±0.11^{bc}	14.38^{d}	8.34^{d}	5.46^{e}
18	1000	5.0	20	12	68.00^{c}	1.47±0.13^{d}	11.85^{d}	8.18^{d}	4.94^{f}
k1	6.47	5.63	5.85	5.83					
k2	5.49	5.79	6.29	5.80					
k3	5.40	5.95	5.23	5.74					
R	1.07	0.33	1.06	0.04					
T_{wi}	-	-	-	-	93.00^{a}	1.67±0.28^{b}	30.29^{a}	15.15^{a}	7.26^{a}

Different superscript letters indicate significant differences by one-way ANOVA test (*P <0.05*)

Table 5 GLM univariate analysis

Source	Sum of squares	Degree of freedom	Mean square	*F*-value	Significant
Factor A	4.268	2	2.134	119.549	<0.001
Factor B	0.323	2	0.162	9.060	<0.05
Factor C	3.416	2	1.708	95.676	<0.001
Factor D	0.026	2	0.013	0.724	0.511
Error	0.161	9	0.018		

P <0.05 indicates significant differences, P <0.001 indicates extremely significant differences

Figures

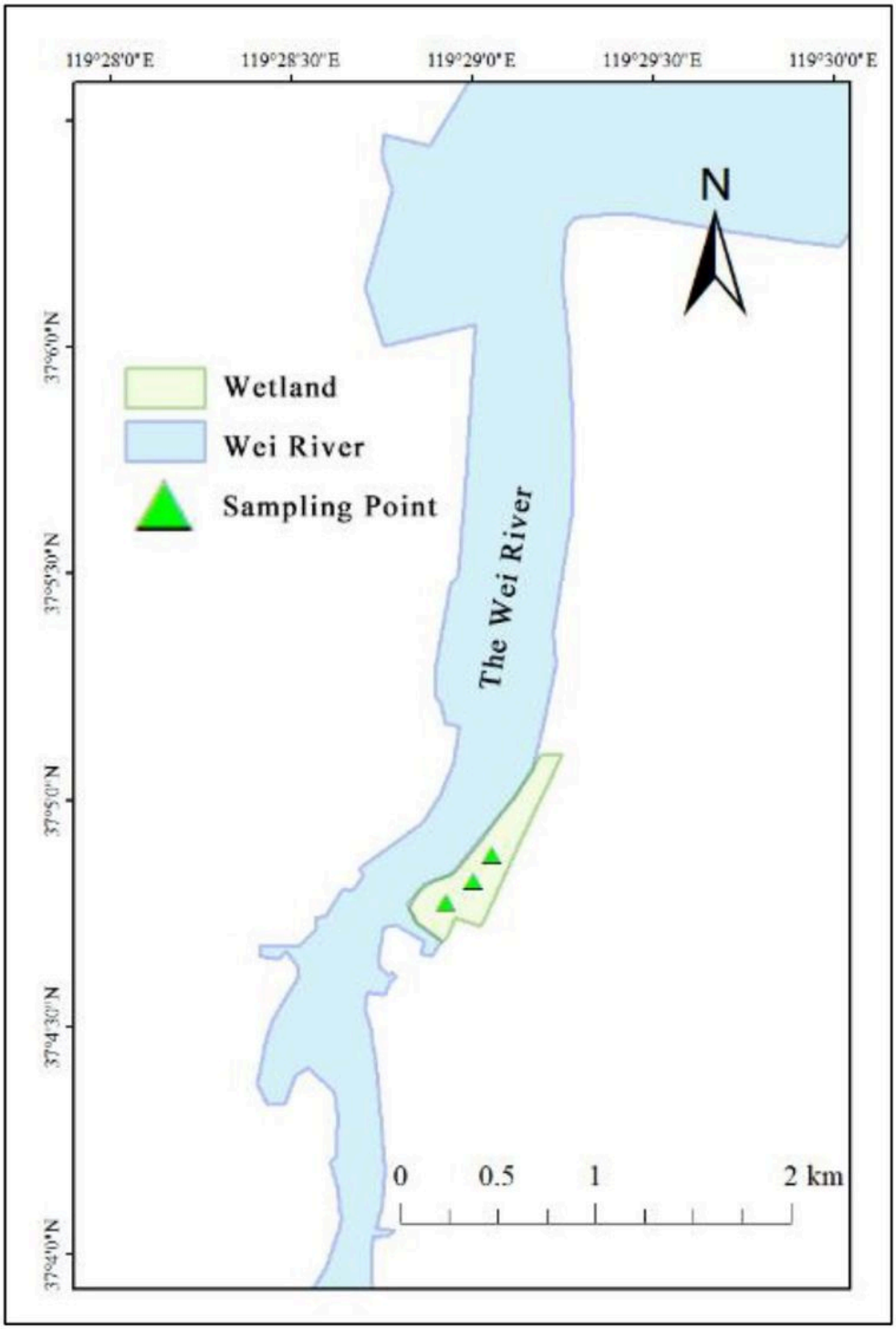


Figure 1

Natural wetland sampling site.

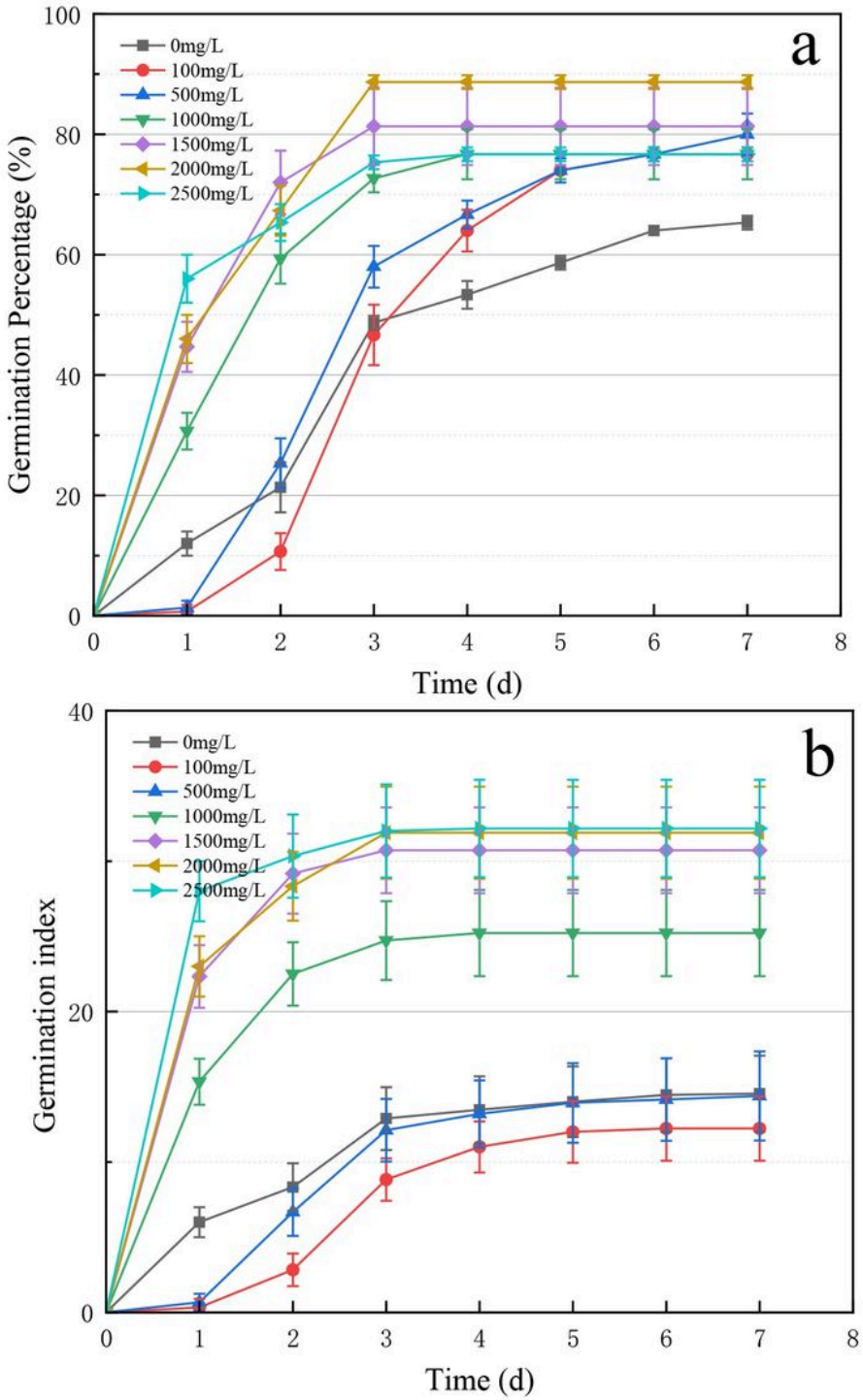


Figure 2

Trends in germination under GA_3 treatment

a shows the trend of germination rate and b shows the trend of germination index.

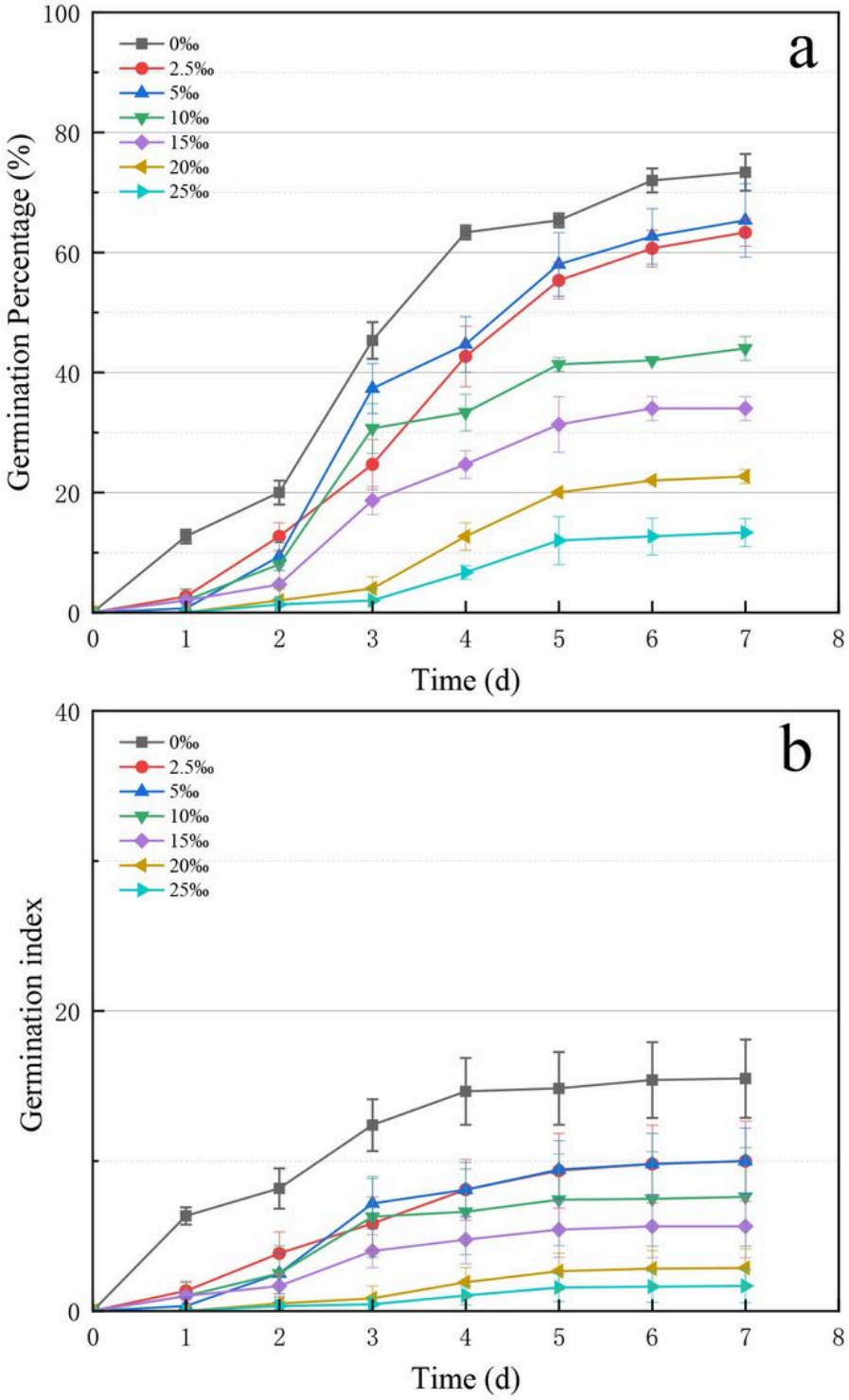


Figure 3

Trends in germination under salinity treatments

a shows the trend of germination rate and b shows the trend of germination index.

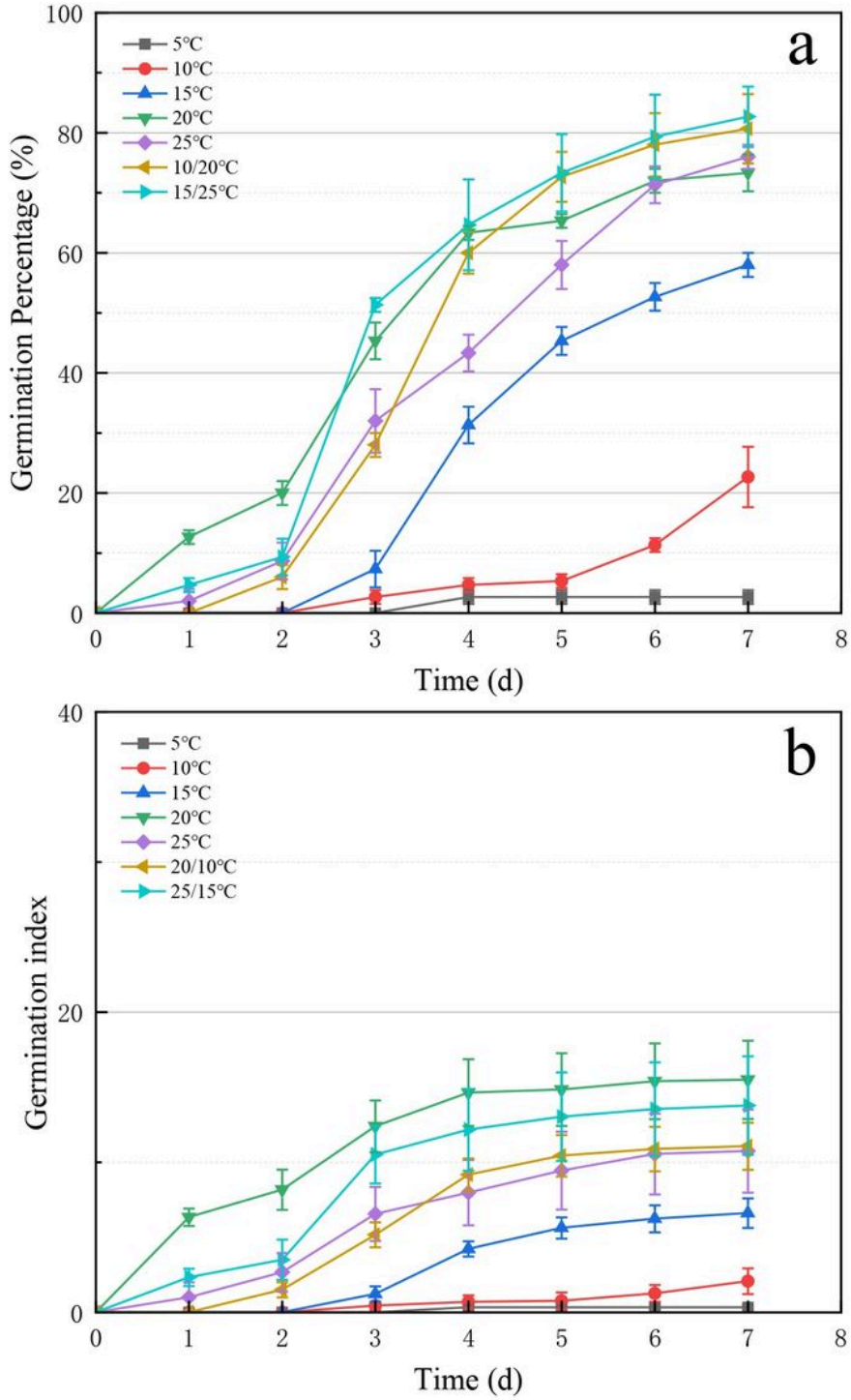


Figure 4

Trends in germination under temperature treatments

a shows the trend of germination rate and b shows the trend of germination index.

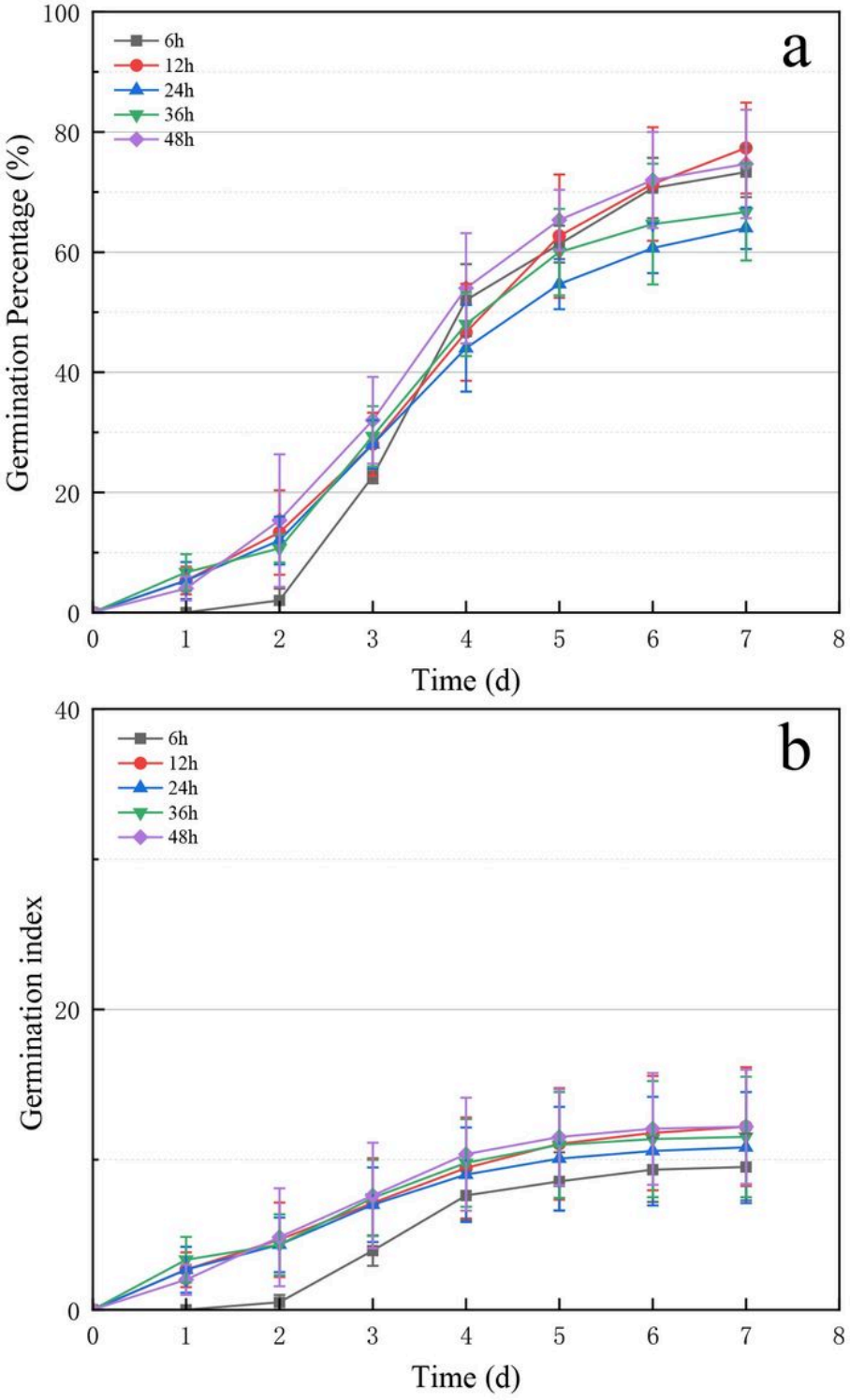


Figure 5

Trends in germination under soaking time treatments

a shows the trend of germination rate and b shows the trend of germination index.

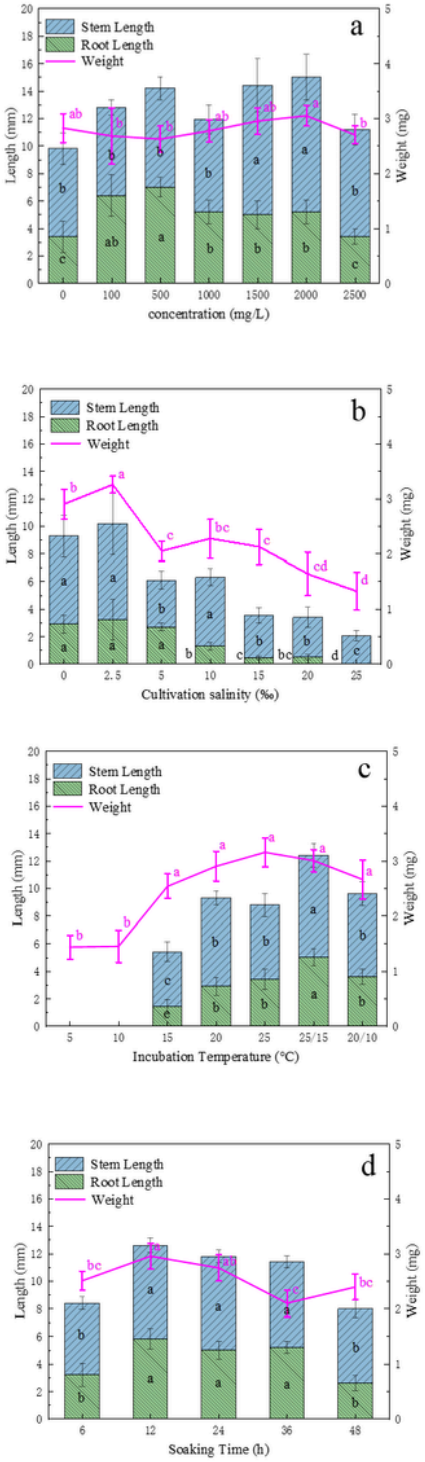


Figure 6

TL and *Wt* development during germination stage

a, b, c and d represent the comparison of *TL* and *Wt* under GA_3 concentration treatment, culture salinity treatment, culture temperature treatment and soaking time treatment, respectively. Single factor analysis

of variance was performed for the same group indicators, and different English letters in the figure represent significant differences (P<0.05).

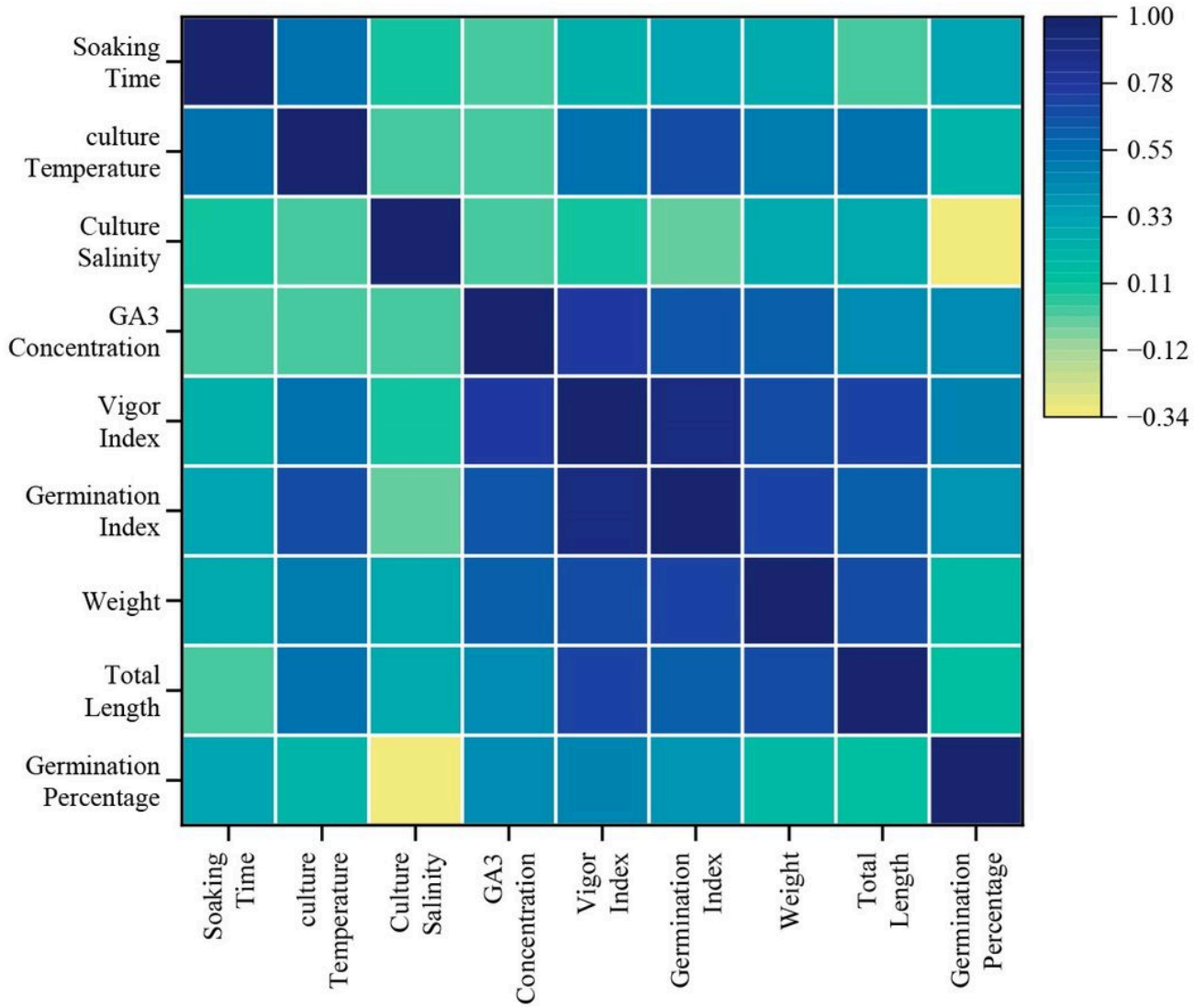


Figure 7

Heatmap of correlation analysis of abiotic factors and growth indicators