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Environmental conditions predetermine quality, germination, and innate antioxidants pool in sea oat (*Uniola paniculata* L.) seeds

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Secondary metabolites such as antioxidants are critical components that protect seeds from stress damage during seed development, desiccation, and *ex-situ* storage. Antioxidants are essential determinants of seed quality, longevity, and persistence. Understanding the environmental factors that regulate the accumulation, content, and function of antioxidant pools in sea oat seeds is critical for gene banking and understanding the environmental impacts on seed quality. Germination, viability, and Trolox Equivalent Antioxidant Capacity (TEAC) were analyzed on seeds from 18 sea oat populations from the US Atlantic and Gulf of Mexico coasts. We first assessed baseline TEAC, followed by TEAC performed on imbibed seeds for 36 h at 35/25°C during the day and night, respectively. Then, we analyzed the relationship of the antioxidant pools from these 18 sea oat populations to sampling site environmental classifications. Higher baseline antioxidants were common in regions with extreme environmental conditions corresponding to sea oat populations growing at latitudinal extremes characterized by warmer and colder temperatures. Baseline antioxidants did not correlate with prevailing seed germination. However, higher concentrations of antioxidants following imbibition were associated with poor seed germination in warmer conditions. Our results indicate that climatic conditions and environmental components associated with temperatures and precipitation may largely influence the innate pool of antioxidants in sea oat seeds. Also, a high amount of antioxidants following sea oat seed imbibition suggest seed damage or poor viability influenced by environmental stress conditions during seed development.

KEYWORDS

climate, environmental gradients, germination, latitude, seed ecophysiology

Introduction

Uniola paniculata L. (sea oats [Poaceae]) is an ecological keystone species occurring in coastal dune systems throughout the Yucatan peninsula, Cuba, the Bahamas, and southeastern US coastlines (Subudhi et al., 2005; Lonard et al., 2011; Hodel and Gonzales, 2013). Sea oats are essential for their ecological services, such as sand accretion, preventing erosion, and forming and stabilizing coastal dunes. These services

largely buffer the effect of extreme events such as storms and hurricanes prevalent along those coastlines (Lonard et al., 2011; Hacker et al., 2019). Consequently, sea oats are in high demand for habitat restoration and re-vegetation programs. However, the spatial extent of sea oats populations has diminished greatly due to habitat destruction, conversion, and fragmentation. Protecting remaining sea oats populations is therefore crucial for maintaining donor populations for seed-based conservation and restoration programs.

Sea oats face diverse extreme conditions in the coastal dune habitat including osmotic stress from aerosolized salts and saline soils, hypoxia due to flooding, high and low-temperature stress, high humidity stress, and physical damage from tidal and wave over-wash or winds caused by storms and hurricanes. Sea oats also face burial from shifting dune sands. Therefore, sea oats and their seeds need highly functional stress-relief systems, especially antioxidants, to survive such conditions. However, there is limited knowledge on the presence, environmental influence, and functional role of antioxidants in plants from coastal dune systems exposed to such wide-ranging stressors.

Antioxidants are a category of secondary metabolites synthesized by living organisms that have been associated with preventing oxidative damage to cells and tissues. These molecules function at relatively low concentrations during optimal and stress conditions (Halliwell et al., 1995; Begcy et al., 2012) directly impacting complex aspects of seed quality including nutritional components, seed size, water potential and dry matter content (Bailly, 2004, 2019; Corbineau, 2012). In seeds, antioxidants are synthesized throughout the developmental program (i.e., histodifferentiation, reserve accumulation, and late maturation) and are thought to protect against stresses by quenching reactive oxygen species (ROS) and free radicals (Bailly, 2004; Chen et al., 2016; Adetunji et al., 2021). For instance, antioxidants balance ROS and free radicals generated in hydration-associated active metabolism from the initiation of embryogenesis through germination by acting as free radical scavengers (Pehlivan, 2017). Seed-based antioxidants also influence the hydration process through the action of seed coat phenolics, which may offer a barrier to rapid imbibition (Ross et al., 2010). They also regulate cellular redox balance preventing oxidative damage during the imbibition process (Bailly, 2004). Antioxidants also substantially buffer the damage from ROS and other radicles generated by desiccation stress and lipid oxidation during seed storage (Groot et al., 2015; Carta et al., 2018; Adetunji et al., 2021; Stegner et al., 2022). Furthermore, antioxidants may be linked to maintaining seed persistence and viability in the soil seed bank (Long et al., 2015).

At an ecological level, plants adapt to prevailing environmental conditions such as precipitation, relative humidity, solar radiation, and temperatures, allowing normal plant development and reproductive success (Saatkamp et al., 2019). Such adaptations result in the development of fitness-related traits vital to surviving harsh conditions such as drought and cold stress (Capblancq et al., 2022; Eckert and Neale, 2022). Studies have indicated a strong correlation between environmental conditions and plant traits. For example, the influence of geographical location on seed germination has been observed in sea oats (Pérez and Kane, 2017). At the same time, environmental conditions during plant growth, flowering, and seed development has been found to influence subsequent seed germination and seedling response to oxidative stress (Nguyen et al., 2021).

Seed traits including seed dormancy and vigor as well as resilience to environmental stresses have been suggested to vary broadly across a latitudinal gradient (Baskin and Baskin, 2014; Pérez and Kane, 2017; Moreira et al., 2020; Zhou et al., 2021). Using sea oat populations distributed across the US Atlantic and Gulf Coastlines, we investigated the functional dynamics of antioxidant pools in sea oats seeds collected from a continental-scale spatial distribution. First, we measured their antioxidant levels hypothesizing possible interactive links of the environmental conditions to the antioxidant levels and seed quality. We compared antioxidant levels from each population to 10-year climate data (precipitation, and temperature) and various ecological classification systems of the study sites. We further tested the potential role of the antioxidant pool on seed quality by correlating antioxidant levels to germination ability.

Materials and methods

Plant material collection and seed lot processing

We collected one to three sea oats (*Uniola paniculata* L.) panicles from no less than 30 widely spaced plants across 18 sites along the US Atlantic and Gulf of Mexico (referred to hereafter as Gulf) coasts in October 2019 (Figure 1A, Table 1). We spread panicles in a single layer on tarps within a non-climate-controlled warehouse and circulated air over the panicles with an electric fan. We allowed panicles to dry for 3 days, then hand-stripped spikelets and threshed caryopses (referred to hereafter as seeds) from spikelets with a laboratory debarker (Heavy Duty Batch Debarker, DB3001-600, Mater Seed Equipment, Corvallis, OR). We conditioned seeds further by passing all lots through an air-screen cleaner (Clipper Office Tester, A.T. Ferrell Co. Inc., Corvallis, OR) then an air-column separator (Oregon Seed Blower, Hoffman Manufacturing Inc., Corvallis, OR). We stored remaining seeds in the lab for two and half years (~23°C, 30%–50% RH).

Seed imbibition and germination assays

We randomly selected 200 seeds from each population for imbibition and germination assays. We surface sterilized tested seeds by placing them in 250 mL glass conical flasks with sufficient ethanol (50% v:v) to cover all the seeds (ca., 40 mL). The flasks were transferred to a benchtop shaker and agitated at 200 rpm for 10 min. This was followed by a double rinse (i.e., 3 min each) with 100 mL of sterilized distilled H₂O followed by adding 40 mL of 25% sodium hypochlorite and incubation on a benchtop shaker at 200 rpm for 5 min, then another double rinse with 100 mL of sterilized, distilled H₂O (i.e., 3 min each) before using them in imbibition and germination assays (Begcy et al., 2018).

We performed seed imbibition experiments by placing fifteen seeds on blotter paper (Steel Blue, Anchor Paper Co., St. Paul, MN) moistened with 30–50 mL distilled H₂O containing 0.2% solution of a broad-spectrum biocide (Plant Preservative Mixture, Plant Cell Technologies, Washington, D.C.) contained within polystyrene germination boxes (156C, Hoffman Manufacturing, Corvallis, OR). We then transferred boxes to an incubator (I30-VL,

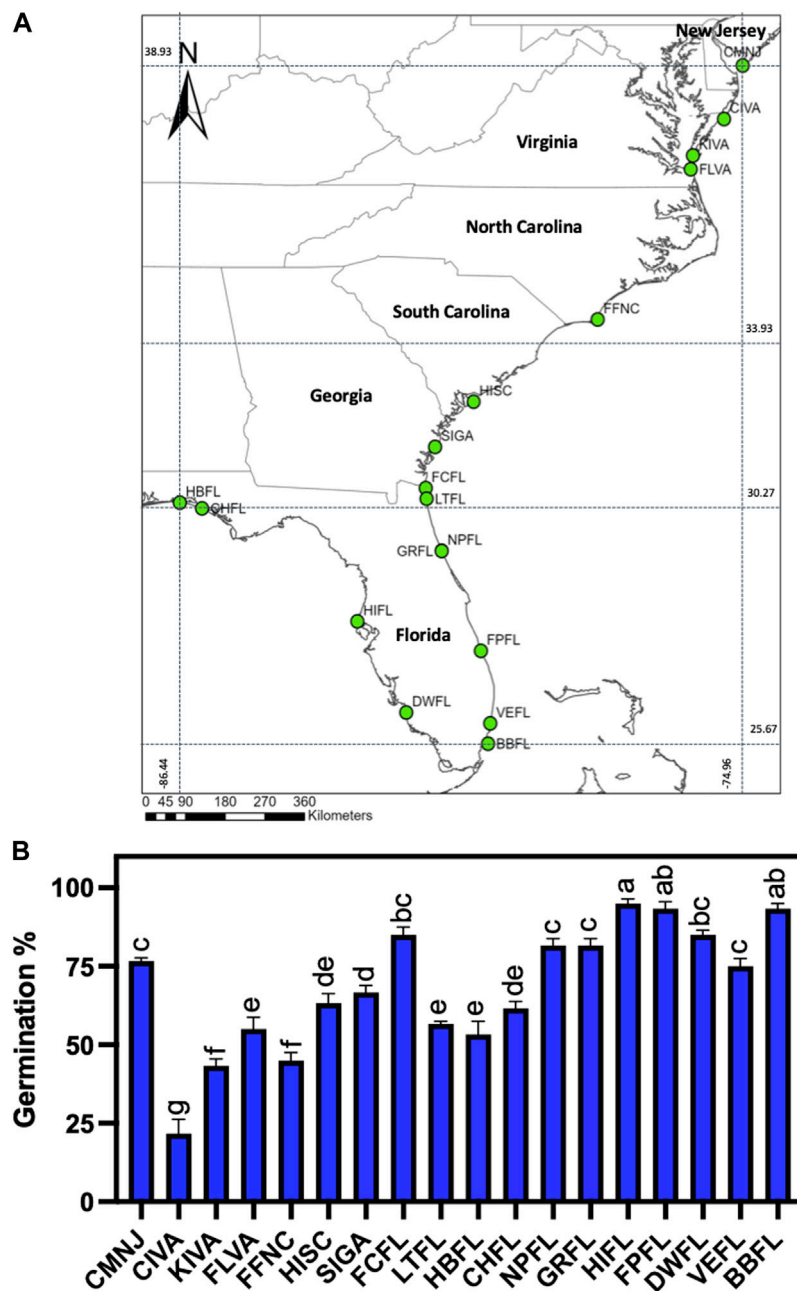


FIGURE 1

Sea oats seed collection sites and germination from 18 populations harvested in the US Atlantic and Gulf Coastlines. (A) Continental-scale map showing the collection sites representing different sea oat populations. CMNJ, Cape May Plant Materials Center; CIVA, Chincoteague National Wildlife Refuge; KIVA, Kiptopeke State Park; FLVA, First Landing State Park; FFNC, Fort Fisher State Recreation Area; HISC, Hunting Island State Park; SIGA, Sapelo Island National Estuarine Research Reserve; FCFL, Fort Clinch State Park; LTFL, Little Talbot Island State Park; NPFL, North Peninsula State Park; GRFL, Gamble Rogers State Park; FPFL, Fort Pierce Inlet State Park; VEF, Dr. Von D. Mizell-Eula Johnson State Park; BBFL, Bill Baggs State Park; HBFL, Henderson Beach State Park; CHFL, Camp Helen State Park; HIFL, Honeymoon Island State Park and DWFL, Delnor-Wiggins State Park. North Peninsula State Park and Gamble Rogers State Park are close together and appear as a single population on the map. (B) Sea oat seed germination. Error bars indicate the standard error of the mean. Seed germination test was performed using 25 seeds in four independent replicates from a random sample of 200 seeds obtained from the seed stock of more than 500 seeds. Seed germination was based on the percentage of germinated seeds sown determined by the exposure of 2 mm of radicle and coleoptile emergence at 28 days. Collection sites are displayed left to right based on their latitudinal location.

Percival Scientific, Perry IA) set to 35/25°C (day/night) and a 12 h photoperiod. Illumination coincided with the day temperature. We allowed seeds to imbibe under these conditions for 36 h, after which we froze the seeds in liquid nitrogen and stored them at -80°C for

use in the determination of Trolox Equivalent Antioxidant Capacity (TEAC) as described below.

We used three to four samples of 25 seeds in our germination experiments that were conducted under the same conditions

TABLE 1 Sea oats seed collections sites from the US Atlantic and Gulf Coastlines.

Code	Population	State	Longitude	Latitude
	Atlantic Coast Populations			
CMNJ	Cape May Plant Materials Center	New Jersey	-74.961948	38.93669
CIVA	Chincoteague National Wildlife Refuge	Virginia	-75.3454413	37.8908169
KIVA	Kiptopeke State Park	Virginia	-75.9747921	37.1735949
FLVA	First Landing State Park	Virginia	-76.019084	36.9055266
FFNC	Fort Fisher State Recreation Area	North Carolina	-77.922256	33.962983
HISC	Hunting Island State Park	South Carolina	-80.4521216	32.3585481
SIGA	Sapelo Island National Estuarine Research Reserve	Georgia	-81.2408689	31.4764158
FCFL	Fort Clinch State Park	Florida	-81.434374	30.6679703
LTFL	Little Talbot Island State Park	Florida	-81.4159693	30.4600485
NPFL	North Peninsula State Park	Florida	-81.109324	29.441617
GRFL	Gamble Rogers State Park	Florida	-81.1099577	29.4380465
FPFL	Fort Pierce Inlet State Park	Florida	-80.3031879	27.4852057
VEFL	Dr. Von D. Mizell-Eula Johnson State Park	Florida	-80.1115753	26.06652
BBFL	Bill Baggs State Park	Florida	-80.1568866	25.6718455
	Gulf Coast Populations			
HBFL	Henderson Beach State Park	Florida	-86.4465757	30.3851647
CHFL	Camp Helen State Park	Florida	-85.9922708	30.2732725
HIFL	Honeymoon Island State Park	Florida	-82.8263531	28.0603784
DWFL	Delnor-Wiggins State Park	Florida	-81.8279833	26.2816886

described for the imbibition experiments. However, germination assays continued for 28 days. We inspected the germination boxes daily and counted germination when seeds exhibited 2 mm of radicle and coleoptile emergence (Begcy et al., 2018). We removed germinated seeds and any seeds showing signs of fungal contamination. We assessed the viability of any remaining, non-germinated seeds after day 28 using tetrazolium stain and analyzed embryo staining patterns at magnifications of 2.5–6.4× (Supplementary Figures S1A, B) (Peters and Lanham, 2000).

Seed water relations

We measured post-storage seed water potential, using five technical replicates, with a dewpoint potentiometer (WP4C, Decagon Devices, Pullman, WA). The potentiometer was calibrated before each measurement day according to manufacturer recommendations. We measured seed water potential at 20°C in precise mode. In this procedure, we used about 200 seeds to cover the bottom of the cuvette completely. Seed moisture content was calculated by recording the fresh mass on 20 seeds in five replicates for each of the 18 populations studied. We then dried these seeds in a forced-air oven (Blue M, Illinois, United States) at 103°C for 17 h and report water content on a dry mass basis ($\text{g H}_2\text{O} \cdot \text{g dry seed mass}^{-1}$ [g g^{-1}]).

Climate and ecological data

We obtained climate data for the 10 years before our seed sampling (2009–2019) using the PRISM application (Hart and Bell, 2015). The climate data used in this study included minimum (Tmin), mean (Tmean) and maximum (Tmax) temperatures, mean dew point temperature (Tdmean), total precipitation (ppt), and daily maximum vapor pressure deficit (Vpdmax). All data obtained were filtered at 4 km resolution using the location coordinates for sites corresponding to each of the 18 sea oat populations (Figure 1A, Supplementary Table S1). Data were extracted using the prism R package (Hart and Bell, 2015).

First, we explored seasonal precipitation and temperature patterns along the latitudinal gradient where the sea oats populations were collected. We organized our analysis into groups of 3 months, roughly representing the four seasons (Winter, Spring, Summer, and Autumn).

Ecological descriptors comprised the: U.S. Environmental Protection Agency (US EPA) ecoregion zoning (EPA3 and EPA4) (Supplementary Table S2), U.S. Department of Agriculture-Natural Resource Conservation Service (USDA-NRCS) and Food and Agriculture Organization of the United Nations (FAO) soil classification (Supplementary Table S3), American Horticulture Society (AHS) heat zoning (Supplementary Table S4), USDA plant hardiness zoning (Supplementary Table S5). We also used

sea oat phylo-geography data as population grouping factors (Franks et al., 2004, Supplementary Table S6).

Antioxidant analysis

We estimated the levels of Trolox Equivalent Antioxidant Capacity (TEAC) in seeds using the modified ABTS/TEAC assay (Re et al., 1999). ABTS/TEAC determines the TEAC per gram of sample. ABTS (2,2'-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) reagent was prepared by dissolving 2.74 mg of ABTS in 1 mL of sterile, distilled H₂O for each reaction, including the standards. Then, 300 mg of MnO₂ was added and mixed using a magnetic stirrer for 20 min. The mixture was filter sterilized through a 0.45 μm syringe filter. For calculations, a spectrophotometer (Scientific Genesys 10S UV-VIS Spectrophotometer, Thermo Scientific, Madison, United States) was zeroed at 734 nm with 100 μL PBS added to 1 mL of sterile distilled H₂O. Absorbance readings were adjusted with 5 mM PBS to obtain a value of 0.700. We added 6.26 mg of Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) to 50 mL of 5 mM PBS then mixed the reagents by sonication for 20 min to obtain 0.5 mM Trolox standard solution. Then, 1 mL of aliquots were prepared in cryovials and stored in a freezer at -20°C. During the assay, a 1 mL cryovial containing 0.5 mM Trolox was obtained from the freezer and allowed to thaw. We re-dissolved the solution by sonication for 10 min. Then, dilutions for five triplicate standards were obtained at 0, 11, 22, 33, and 44 μL of 5 mM Trolox corresponding to TEAC concentrations of 0, 5, 10, 15, and 20 μmol/L, respectively.

Antioxidants for TEAC estimation were extracted from seeds using a modified hydrophilic method (Re et al., 1999). Samples were finely ground using liquid nitrogen. Approximately 10 mg/sample was placed into 10 mm × 75 mm reaction vials. Triplicates were used per sample per reaction. TEAC was extracted by adding 5 mL of 75% aqueous methanol, followed by stirring under a nitrogen gas stream of 2.0 Pascals at 30°C for 60 min. Then, the obtained product was centrifuged at 2000 × g for 5 min to get a supernatant containing the extracts. A second extraction step was conducted by adding 2 mL of 75% aqueous methanol and covering the reaction vials. Then, each vial was vortexed and centrifuged at 2000 × g for 15 min. Supernatants of the first and second reactions were combined. Concurrently, 10–20 mg of finely ground samples were prepared in oven-drying foil pans and dried at 103°C for 17 h to obtain dry mass.

Extracts were re-suspended into 10 mL of 75% methanol. Each 100 μL of sample extract was mixed with 1 mL of 5 mM ABTS solution, vortexed for 2 min, and placed in the spectrophotometer cuvette before reading the absorbance at 734 nm. Three biological replicates and three technical replicates were used.

Data analysis

We subjected data on seed water potential, moisture content, TEAC, and germination percentage to an analysis of variance (ANOVA) to estimate differences among populations. We utilized linear and non-linear polynomial regressions to evaluate

seed germination and TEAC levels against geographical, climatic, and ecological patterns. When linear regression analysis was used, we estimated the coefficient of determination (R^2) using the Pearson correlation coefficients (r). For linear regression using ordinal data, we performed a non-parametric test for the significance of the correlation using the Spearman correlation to estimate the Spearman's rho (r_s) and the associated approximate p -value to test for the presence of monotonic relationships as previously described (Kvalseth, 1985). For non-linear regressions, the model selection was based on Akaike information criterion (AIC) involving the comparison of the best non-linear model to an alternative linear model. We tested the selected non-linear model by comparing to a "null model" for estimating the statistical significance using the "rcompanion" package in R software version 4.2.3 following the methods described previously (Mangiafico, 2015). We represent the overall p -values and the pseudo R^2 (Nagelkerke R-squared) values (Mangiafico, 2015) for these non-linear models. Statistical analyses were performed using the R software/environment versions 4.1.1 and 4.2.3 (R Core Team, 2021). Differences were considered statistically significant at p -value < 0.05 (Kim et al., 2021).

Results

Impact of the latitudinal gradient on seed physiology in sea oat populations along the US Atlantic and Gulf coastlines

To test the hypothesis of seed related traits variation across a wide latitudinal gradient (Baskin and Baskin, 2014; Pérez and Kane, 2017; Moreira et al., 2020; Zhou et al., 2021), we collected and germinated eighteen sea oat populations distributed across the US Atlantic and Gulf Coastlines (Figure 1A). Seed germination ranged from 20% (CIVA) to 95% (HIFL) (Figure 1B). Seeds from 39% of the populations registered germination >80%, most of the populations displaying this level of germination were from Florida (Figure 1B). However, there were instances of poor germination (i.e., <50%) for other populations, especially from the northern latitudes of the Atlantic coastline (Figure 1B). Interestingly, seeds collected from populations in the southern US displayed germination percentages > 55% (Figure 1B).

To further understand differences in seed physiology that may influence the variation of germination, we quantified seed moisture content and water potential (Figures 2A, B). Seed moisture content was relatively similar across the latitudinal gradient and ranged from 0.1064 to 0.1129 g g⁻¹. Seeds with lower moisture content (<0.1100 g g⁻¹) comprised KIVA, FFNC, SIGA, GRFL, NPFL, and HBFL (Figure 2A). While seeds collected from LTFL and CIVA displayed the highest water contents observed of 0.1129 and 0.1130 g g⁻¹, respectively. The seeds with the lowest moisture content were collected from Florida. They included GRFL and NPFL at 0.1066 and 0.1064 g g⁻¹, respectively (Figure 2A). Sea oats seed water potential was also variable and ranged from -89.01 to -100.84 MPa (Figure 2B). Patterns of water potential did not necessarily track with patterns of water content as some populations displaying lower water content expressed higher water potential values. Conversely, other populations displaying higher water contents displayed lower

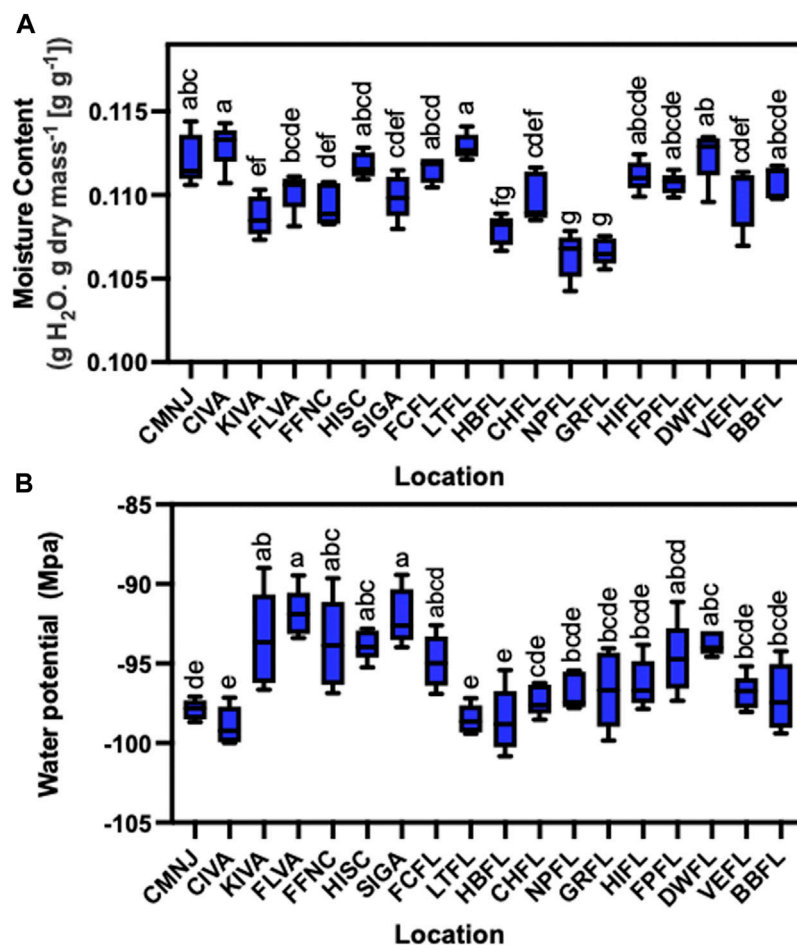


FIGURE 2

Limited variation on moisture content and water potential of sea oats seeds collected across the US Atlantic and Gulf Coastlines. Box plots showing (A) moisture content ($\text{g H}_2\text{O g}^{-1}$). The seed fresh mass was measured on 20 seeds in five independent replicates for each of the 18 populations studied and then dried in a forced-air oven (Blue M, Illinois, United States) at 103°C for 17 h and measured afterward to obtain the seed dry mass ($n = 5$). (B) Water potential (Mpa). Seed moisture content was calculated by obtaining the difference between fresh and dry mass and expressing the value on seed dry mass basis. Seed water potential was estimated from 200 randomly selected seeds from the seed stock covering the bottom of the cuvette completely and measured in precise mode using a dewpoint potentiometer (WP4C, Decagon Devices, Pullman, WA) in five independent replicates ($n = 5$). A different letter indicates significant differences based on one way analysis of variance (ANOVA) at an alpha of 0.05.

water potentials (Figures 1B, 2A, B), and we did not find correlations between these two seed water relation traits with the seed germination traits (Supplementary Figure S2). Therefore, seed water relations could not explain germination variation across populations.

To characterize the extent to which location and environmental conditions influenced the germination of sea oats seeds, we examined how germination related to latitude and temperature zoning (Figure 3; Supplementary Tables S4, S5). We observed a latitudinal pattern of germination response, with regions closer to the equator having higher germination while populations located further from the equator displayed reduced germination capacity ($R^2 = 0.49$), indicating a significant latitudinal influence on germination ability (Figure 3A). In addition, germination was lower in zones with fewer heat days above 30°C but increased as the number of heat days increased (Figure 3B). Similarly, germination was lowest in zones with lower

average minimum winter temperatures but increased as this average rose (Figure 3C).

Weather characteristics of sea oat seeds collection sites along the US Atlantic and Gulf coastlines

To explain which factors could have contributed to the observed differences in seed traits across all sea oats populations (Figures 1–3), we first looked at precipitation and seasonal mean temperature patterns for sea oats collection sites along the US Atlantic and Gulf coastlines in a 10-year period prior to seed sampling (2009–2019). Precipitation patterns across the latitudinal gradient were similar in winter and spring, averaging 50–150 mm (Figure 4A). However, in summer, southerly latitudes displayed higher precipitation (>150 mm) than northerly latitudes (<150 mm). In Autumn, the

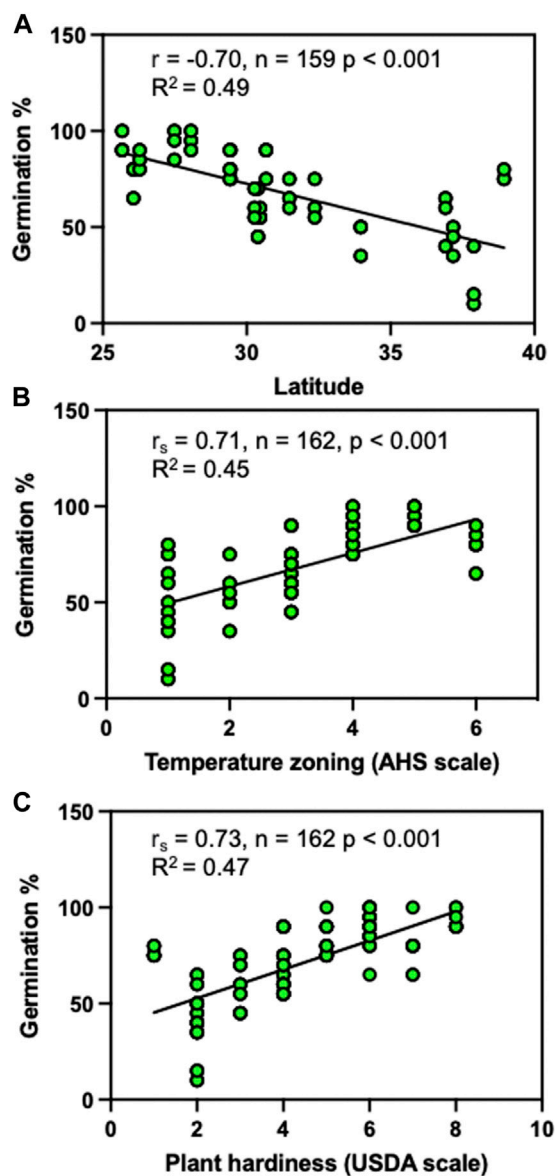


FIGURE 3

Influence of location and environmental zoning by temperature factors on the germination of sea oat seeds. (A) Latitude, (B) AHS temperature zoning, and (C) USDA plant hardiness zoning. The AHS temperature zoning is based on days at temperatures above 86°C, covering 45–60 days (1), 60–90 days (2), 90–120 days (3), 120–150 days (4), 150–180 days (5) and 180–210 days (6) per year. USDA plant hardiness scale is based on vegetation exposure to cold conditions in winter, at levels of 1(0–10F), 2(10–15F), 3(15–20F), 4(20–25F), 5(25–30F), 6(30–35F), 7(35–40F) and 8 (40–45F). For linear regressions, we estimated the coefficient of determination (R^2) using the Pearson correlation coefficients (r) for continuous data (A) and performed a non-parametric test for the significance of the correlation using the Spearman correlation to estimate the Spearman's rho (r_s) and the associated approximate p -value to test for the presence of monotonic relationships for the analysis involving ordinal data (B,C).

southerly latitudes maintained higher precipitation in September and October (>100 mm) before dropping below 100 mm in November. Interestingly, precipitation was much higher in southern than northern latitudes in September, while higher

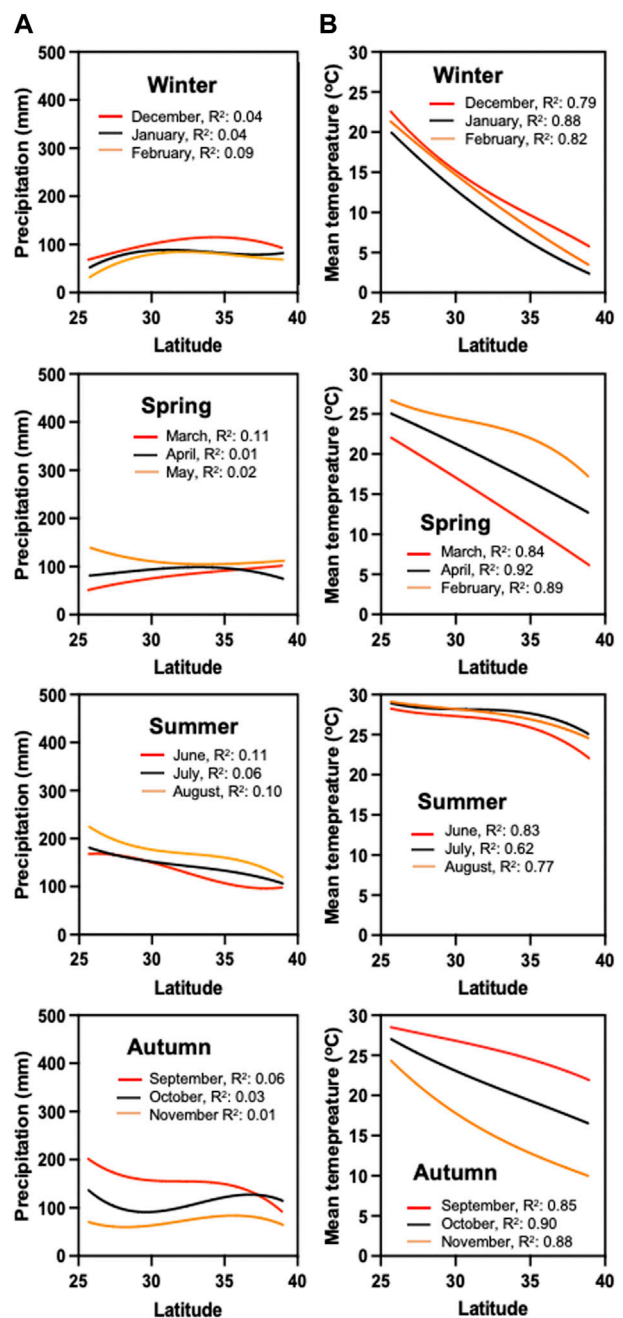


FIGURE 4

Environmental data patterns for the sites of sea oats collection from the US Atlantic and Gulf coastlines in a 10-year period prior to seed sampling (2009–2019). (A) Precipitation and (B) seasonal mean temperature. Model for precipitation and temperature patterns follow the function $Y = B_0 + B_1 + B_2x^2 + B_3x^3$. The coefficient of determination (R^2) indicates the fitness of non-linear regression.

precipitation in the north was observed in October > ca.100 mm (Figure 4A).

Temperature patterns were similar across all seasons (Figure 4B). However, higher temperatures were evident in southern latitudes compared to the northern ones during the Winter, Spring, and Autumn (Figure 4B). Summer temperatures varied less across all the latitudes in which the sea oats populations

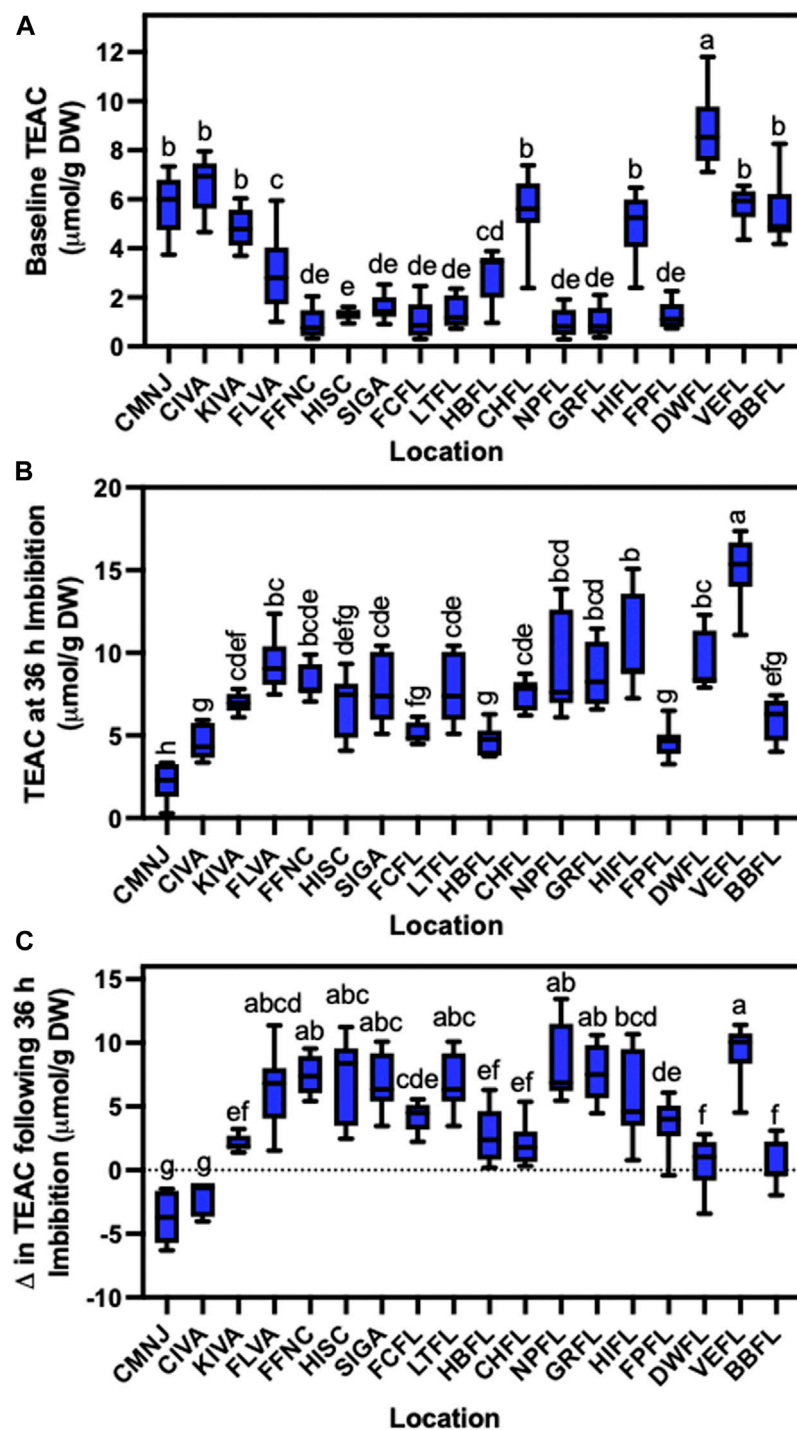


FIGURE 5

Baseline antioxidant levels of dry seeds and after 36 h of imbibition collected from a continental-scale spatial distribution. Baseline TEAC on (A) dry seeds, (B) after 36 h of imbibition, and the (C) difference in TEAC between dry seeds and after 36 h of imbibition. The Trolox Equivalent Antioxidant Capacity (TEAC) was extracted using the modified ABTS/TEAC assay in three biological replicates and three technical replicates. Different letters code based on one way analysis of variance (ANOVA) at an alpha of 0.05 indicate significant differences ($n = 3$).

were analyzed (Figure 4B). Mean temperatures across all latitudes remained above 22°C throughout June, July, and August (Figure 4B). Both the maximum vapor pressure deficit and the mean dew point had a similar pattern, with higher values in the southern latitudes

and lower values in the northern latitudes in most seasons (Winter, Spring, and Autumn), except in summer season in which there were no differences between southern and north latitudes (Supplementary Figures S3, S4).

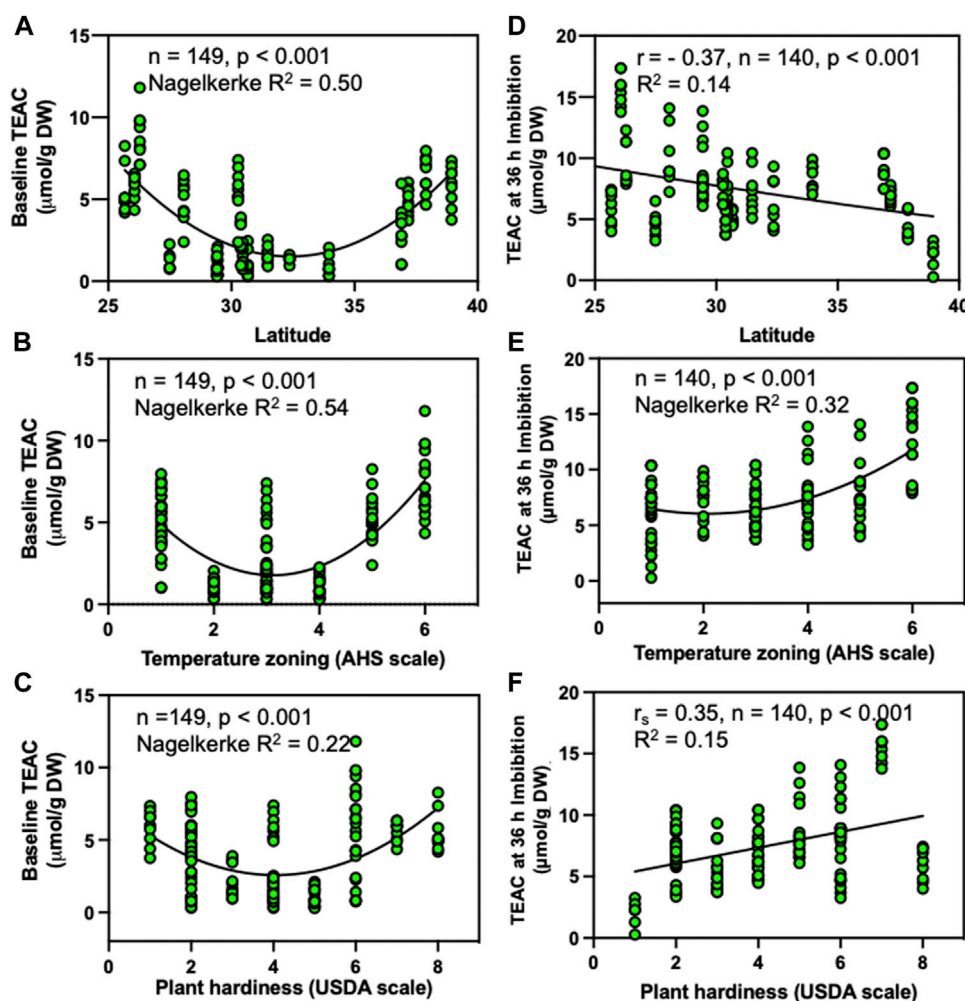


FIGURE 6

Influence of location and environmental zoning by temperature factors on antioxidant levels. Relationship of TEAC in dry seeds (A–C) and after 36 h of imbibition (D–F). (A–D) Latitude, (B–E) AHS temperature zoning, and (C–F) USDA plant hardiness zoning. The AHS temperature zoning is based on days at temperatures above 86°C, covering 45–60 days (1), 60–90 days (2), 90–120 days (3), 120–150 days (4), 150–180 days (5) and 180–210 days (6) per year, while the USDA plant hardiness scale is based on vegetation exposure to cold conditions in winter, at levels of 1(0–10F), 2(10–15F), 3(15–20F), 4(20–25F), 5(25–30F), 6(30–35F), 7(35–40F) and 8(40–45F). In the non-linear models (A–C, E), we represent the overall p values and the statistical significance (Nagelkerke R -squared values) obtained by comparing the non-linear model to a “null model” as described previously (Mangiafico, 2015). For linear regression with continuous data (D), we estimated the coefficient of determination (R^2) using the Pearson correlation coefficients (r). For the linear regression using ordinal data (F), we used the Spearman correlation to estimate the Spearman’s rho (r_s) and the associated approximate p -values.

Latitude-dependent environmental conditions influence TEAC concentration in sea oats seeds

Since our climate data pointed to precipitation and temperature as main drivers of germination differences across a latitudinal gradient except in Autumn (Supplementary Tables S7–S10), we used antioxidant levels in sea oats seeds as a proxy to explore a possible link of latitude-dependent environmental conditions with antioxidant accumulation and germination. First, we defined baseline TEAC values obtained from mature ungerminated seeds before imbibition across all sea oats populations. We found that TEAC levels from our collected seeds ranged from 0 to 12 $\mu\text{mol/g DW}$ (Figure 5A). For instance, while seeds collected in CMNJ, CIVA, KIVA, VEFL, BBFL, HIFL DWFL and CHFL showed TEAC levels higher than 4 $\mu\text{mol/g}$, the remaining populations

showed lower values than that (Figure 5A). Interestingly, in general, seeds from sea oat populations collected either from the most northern or southern locations showed higher antioxidant capacity than the ones collected in intermediate latitudes (Figure 5A). These results suggest an influence by extreme conditions experienced at northern or southern US latitudes. To further explore whether antioxidant capacity was maintained during the initiation of germination, we imbibed seeds for 36 h and quantified their TEAC levels (Figure 5B). Contrary to the earlier patterns of baseline antioxidant levels, there was wider population variability across the geographical distribution of sea oats (Figures 5A, B). The change in TEAC levels between dry and imbibed seeds showed considerable variation (Figure 5C). For instance, seeds from some latitudes displayed a drastic drop in TEAC levels, especially CMNJ and CIVA (Figure 5C), and those with significantly minimal changes comprised KIVA, FPFL, BBFL, DWFL, HBFL, and CHFL

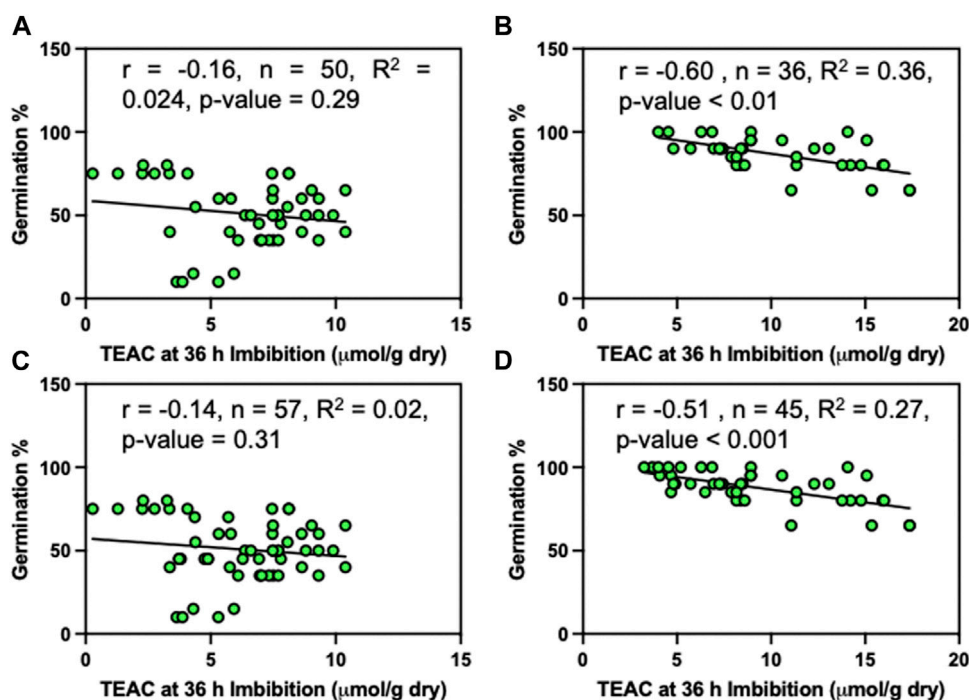


FIGURE 7

Influence of TEAC levels on germination of sea oats seed populations. (A) AHS Temperature zone ≤ 2 ; (B) AHS Temperature zone > 4 ; (C) USDA plant hardiness zone ≤ 3 and (D) zone ≥ 6 . These zones correspond to latitudinal locations of $>33N$ (A,C) and $<30N$ (B,D). The AHS temperature zoning is based on days at temperatures above $86^{\circ}C$, covering 45–60 days (1), 60–90 days (2), 90–120 days (3), 120–150 days (4), 150–180 days (5) and 180–210 days (6) per year, while the USDA plant hardiness scale is based on vegetation exposure to cold conditions in winter, at levels of 1(0–10F), 2(10–15F), 3(15–20F), 4(20–25F), 5(25–30F), 6(30–35F), 7(35–40F) and 8 (40–45F).

(Figure 5C). However, some seeds registered a significantly positive change in TEAC $> 5 \mu\text{mol/g DW}$ comprised of FLVA, FFNC, HISC, SIGA, FCFL, LTFL, GRFL, NPFL and VEFL (Figure 5C).

Geographic and environmental patterns in relation to seed traits in sea oats from US Atlantic and Gulf coastlines

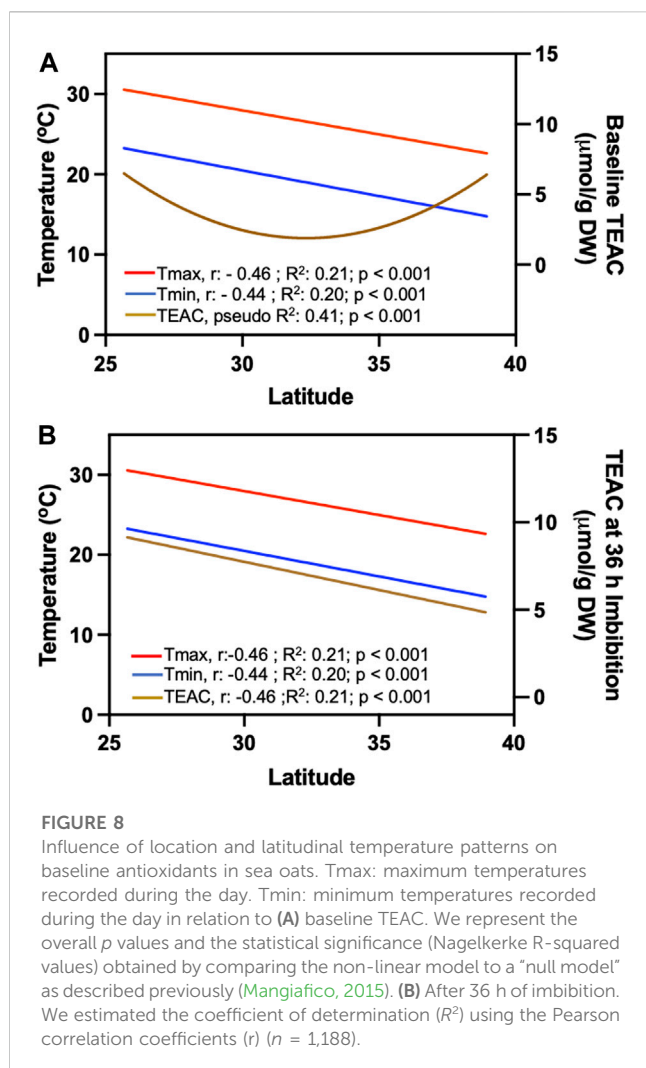
To assess the impact of latitude on antioxidant capacity, we used baseline seed TEAC concentrations and compared these with the latitudinal range where seeds were collected. In general, antioxidant levels in dry seeds displayed a concave parabolic pattern when evaluated against latitude, heat, and plant hardiness zones (Figures 6A–C). Then, we compared antioxidant capacity levels obtained 36 h after imbibition with the latitudinal zones (Figure 6D). We observed higher TEAC levels near the equator and lower levels in northern latitudes. Surprisingly, relationships between TEAC and AHS heat or USDA plant hardiness classifications showed similar patterns when TEAC data obtained from seeds imbibed after 36 h (Figures 6E, F). Zones with a greater number of hot days (Figure 6F) and less freezing conditions (Figure 6E) displayed higher TEAC values. We found a strong ($R^2: 0.50$) latitudinal pattern for TEAC obtained from dry seeds compared against latitude (Figure 6A) and temperature zoning (AHS heat) (Figure 6B) as well as on temperature zoning in 36 h imbibed seeds (Figure 6E). We also found considerable patterns when TEAC obtained from dry and

imbibed seeds were compared against USDA plant hardiness (Figures 6C, F) and imbibed seeds against latitude (Figure 6D).

Role of the environment and seed traits on the baseline germination and antioxidants in sea oats from the US Atlantic and Gulf coastlines

No clear relationships between germination and baseline TEAC or TEAC after 36 h of imbibition were evident when considering the geographical range of sea oats used in this study (Supplementary Figures S5A, B). Therefore, we analyzed relationships between germination and TEAC using subsets of data representing the extreme temperature regions where high TEAC levels had been observed. Similarly, no clear relationships existed between germination and TEAC after 36 h of imbibition for seeds originating from colder regions ($>33N$) (Figures 7A, C). However, seeds from warmer regions ($<30N$), where high seed quality was expected, exhibited a significant negative linear relationship of TEAC levels in imbibed seeds after 36 h (Figures 7B–D).

Finally, we compared the influence of latitude and temperature patterns on TEAC levels on dry seeds and seeds imbibed for 36 h (Figure 8). We observed that the minimum and maximum temperatures during the seed development period (Spring and Summer) exhibited high TEAC levels in seeds collected from northern and south latitudes (Figure 8A). As such, the minimum



and maximum temperatures exhibited latitudinal patterns of distribution (R^2 of 0.21 and 0.20, respectively). Contrary, 36 h of imbibition resulted in lower TEAC levels on seeds collected on northern sites (Figure 8B). We further showed in a correlation matrix the intricate nature of likely interdependence and relations of climate variables with seed germination and seed TEAC characteristics (Supplementary Tables S7–S12), indicating that the environmental and climate conditions faced by sea oat populations substantially impact seed quality traits in terms of germination and antioxidant levels.

Discussion

Sea oats are the primary native dune grass used to restore and stabilize beaches and dunes across the southeastern United States. Commercial nurseries have propagated sea oats using field-collected seeds to retain the genetic integrity of source populations. We investigated the effect of climate conditions on sea oats harvested across a wide latitudinal range that spanned more than 13° of latitude (Table 1). Our results show that sea oats seed germination was higher from the sites closer to the equator but decreased as the distance from the equator increased (Figure 1B). The observations of comparative

germination with respect to distance from the equator indicated that the sea oat seeds are of high quality in terms of germination percentage from warmer tropical regions compared to seeds from colder areas. Similar patterns of seed germination were observed between populations located in Southern Italy in comparison with a Spanish core population of *Lavandula multifida* L., a plant species present in the Western Mediterranean Basin (Panuccio et al., 2018).

Environmental and climate influences on seed quality, antioxidants, and implications on seed germination

Antioxidant activity plays a key role in various events of seed life. Antioxidants are produced throughout the seed developmental program and during the germination process. These molecules occur within metabolically active cells, but also in dry tissues, with physiological activity depending on seed moisture status (Vertucci and Farrant, 1995; Walters et al., 2002; Ballesteros et al., 2020). Water potential measurements of dry seeds tested in this study suggest that seeds from all populations fall into the same hydration level (i.e., Hydration level II, ca. -15 – 190 MPa (Vertucci and Farrant, 1995; Walters et al., 2002; Walters et al., 2005) which was like previous studies (Pérez and Kane, 2017). Hydration level II represents a cellular physiological state characterized by enzyme-mediated catabolic reactions, oxidative and peroxidative processes, and formation of free radicals and reactive oxygen species. Existing pools of molecular antioxidants synthesized during the seed developmental program are thought to play a protective role by quenching reactive oxygen species and free radicals in dry seeds (Vertucci and Farrant, 1995; Walters et al., 2002; Walters et al., 2005). We did not observe a clear relationship between TEAC concentration and baseline seed water potential in this study. Nonetheless, seeds from all populations display antioxidant capacity that may be sufficient to mediate aging associated deteriorative reactions to some degree during storage under non-optimal conditions of temperature and relative humidity (Walters et al., 2005). Moreover, it is well documented that environmental conditions directly impact seed quality as well as their desiccation tolerance, nutrient level, storage potential and the accumulation of antioxidants and other related secondary metabolites (Bewley et al., 2013; Pehlivan, 2017; Ellis, 2019; Malovichko et al., 2021).

We observed differences in the accumulation of antioxidant levels in dry seeds compared to imbibed seeds across all populations (Figures 5, 6). Increased antioxidant activity during seed germination is vital in signaling and ROS homeostasis (Bailey, 2004; El-Maarouf-Bouteau and Bailey, 2008). High germination capacity associated with moderate changes in TEAC levels after initial hours of imbibition is expected to be a characteristic of good-quality seeds because it is linked to a low lipid peroxidation (Rogozhin et al., 2001). In our study, seeds from warmer regions with sharp increases in TEAC following imbibition had poor germination compared to those with limited fluctuations in TEAC. This rise in TEAC levels may be a physiological response to prevent seed deterioration and can also be an indicator of poor-quality seeds or seed damage. These results agree with the elevated antioxidant levels (α -tocopherol and tocotrienols) found in flax (*Linum usitatissimum*) (Oomah et al., 1997) and in barley (*Hordeum vulgare*) seeds after elevated temperatures (Roach et al., 2018).

Similarly, a negative correlation between antioxidants (α -tocopherol and β -tocopherols) was associated with poor longevity for rice seeds from temperate regions (Lee et al., 2019). Therefore, the antioxidant variation may have been conditioned by environmental conditions as reported in other plant species.

Latitudinal gradients correlate with antioxidant accumulation

We aimed to answer whether latitudinal gradients have an impact on seed trait performance. Our results showed variation on germination and antioxidant accumulation along the latitudinal gradient (Figures 1B, 6). Interestingly, the latitude-associated geographic distribution of antioxidants in leaves of Bilberry (*Vaccinium myrtillus* L.) was linked to adaptations to prevailing conditions and prevailing abiotic stress associated with a given geographic location (Martz et al., 2010). Similarly, secondary metabolites such as anthocyanidin are strongly controlled by the latitude and geographic origin in *V. myrtillus* fruits (Åkerström et al., 2010). A similar case was reported for blueberries (*Vaccinium ashei* cv. “Brightwell”) in higher altitudes with higher concentrations of flavonoids, phenols, proanthocyanidins, and anthocyanins, among other compounds (Zeng et al., 2020). Our data shows that latitude-associated environmental variables, especially precipitation and temperatures, influence seed germination and antioxidant characteristics. However, other environmental variables that vary along the latitudinal gradient, such as relative humidity, may also impact seed quality hence further research to address such factors on sea oat seed traits will help to answer this question.

In summary, our results show a significant influence on seed physiological performance along a latitudinal gradient with varying environmental conditions in sea oats. Therefore, it is likely that environmental variables dictate antioxidant accumulation in ungerminated seeds and after imbibition. Antioxidants are among the seed storage components critical for persistence, longevity, storage quality, and viability (Long et al., 2015). Therefore, the involvement of the environment in accumulating these secondary metabolites alongside other storage reserves is critical in seed quality. Knowledge of the interaction of associated environmental factors and their interaction is crucial for plants. It can be helpful in guiding the collection of seed materials and seed quality control for storage and use in conservation efforts.

Data availability statement

The raw data supporting the conclusion of this article will be made available by the authors, without undue reservation.

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Author contributions

AE: Data curation, Formal analysis, Investigation, Methodology, Visualization, Writing–review and editing. HP: Conceptualization, Funding acquisition, Supervision, Writing–review and editing. KB: Conceptualization, Funding acquisition, Supervision, Writing–original draft.

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Conflict of interest

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

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Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fenvs.2023.1263300/full#supplementary-material>

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