Effects of Melatonin Priming on *Suaeda corniculata* Seed Germination, Antioxidant Defense, and Reserve Mobilization: Implications for Salinized Wetland Restoration

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Melatonin priming has been widely reported to positively affect seed germination under abiotic stresses. However, there is still a gap in knowledge on how melatonin priming impacts the seed germination and physiological change of wetland plant species. We assessed the effects of different melatonin concentrations on germination characteristics, antioxidant defense, and reserve mobilization of *Suaeda corniculata* seeds. Priming of *S. corniculata* seeds with 50 μM melatonin significantly improved the germination rate, germination speed, germination index, superoxide dismutase and peroxidase activity, and soluble sugar content as compared with the control, and effectively reduced the malondialdehyde content, promoted starch, soluble protein, and fat mobilization. However, the stress tolerance ability of *S. corniculata* seeds was reduced by high melatonin concentration. The structural equation model indicated that the melatonin priming directly affects the seed germination, while also indirectly regulating the antioxidant defense system and reserve mobilization. In conclusion, melatonin priming affects the *S. corniculata* seed germination under salinization stress in a concentration-dependent manner via both direct and indirect regulatory pathways. Insights into these aspects will advance our understanding of how melatonin priming affects *S. corniculata* seed germination and provide invaluable information and technical support for the restoration of salinized wetlands in the Momoge National Nature Reserve.

Keywords: antioxidant defense, melatonin priming, reserve mobilization, salinization stress, seed germination, *Suaeda corniculata*, wetland restoration

INTRODUCTION

Salinization causes major losses in the earth's ecosystem, with nearly 831 million hectares of land undergoing salinization globally (Ma et al., 2015; Zhao et al., 2021a). Salinization is becoming more widespread due to the interactions between the global climate change and anthropogenic hydrological modifications, especially in the wetland ecosystems in arid and semi-arid regions (An et al., 2019; Feghhenabi et al., 2020; Wang et al., 2021). Salinization not only changes the fundamental physicochemical properties of soil, but also negatively affects the seed germination and plant growth by disturbing the physio-biochemical processes including ionic imbalance, oxidative stress, and osmotic stress, thereby reducing the effective wetlands area, thereby resulting in gradual changes or even disappearance of wetland ecosystem services (Tahjib et al., 2018; Wang et al., 2019; Zhang et al., 2019). Therefore, the quick and effective restoration of salinity-degraded wetlands has become an urgent global concern.

Seeds are pivotal propagative organs which carry the genetic information and support the multifunctional evolution in plants (Kettenring and Tarsa, 2020). Employing strategic seed-based approaches in wetland restoration is key to faster and complete recovery of the targeted underlying vegetation structure and composition (James and Carrick, 2016; Zhang M. Y. et al., 2021). Besides being the beginning of the life cycle of higher plants, seed germination is also the most environmentally stress-sensitive stage of the plant's life history (Chen et al., 2021). Previous studies had identified that salinization weakens the seeds' ability to absorb water from the soil as well as inhibits the growth of the seed and the embryo (Li et al., 2019). Moreover, salinization disturbs the delicate balance between the intracellular ROS production and scavenging, and also reduces the ability of reserve mobilization in seeds (Ibrahim, 2016). The seed's stress-amelioration techniques, which can address limiting environmental constraints, are crucial for improving outcomes in degraded salinized wetlands (Kettenring and Tarsa, 2020). In recent decades, various strategies have been adopted to enhance the abiotic stress tolerance of seeds, such as genetic approach, genetic engineering, and plant breeding. However, these methods are complex, time-consuming, expensive, and have biosafety issues (Cao et al., 2019; Johnson and Puthur, 2021). Therefore, the development of a simple, effective, and economical strategy will be significant in improving the salinization stress tolerance of seeds.

Priming is a pretreatment technology which hydrates the seed in specific solutions for initiating specific metabolic processes before germination (Masondo et al., 2018). It is proven to be an effective and safe strategy for addressing the current and future issues of seed germination under abiotic stress conditions (Forti et al., 2021). The priming treatment enhances the inherent tolerance potential of seeds through many mediums, which, based on hormone, have been widely applied in the pretreatment of commercial seeds (Li et al., 2020; Zhang Y. et al., 2021). Melatonin (N-acetyl-5-methoxtryptamine), an endogenous indoleamine, is a highly evolutionarily conserved multifunctional molecule, which is proven to be an abiotic

anti-stress hormone in seeds and is recently being considered as a research hotspot in the priming domain (Li et al., 2016; Arnao and Hernandez-Ruiz, 2019; Zhang T. G. et al., 2022). Recent studies have demonstrated that melatonin priming boosted the activities and transcript levels of antioxidant enzymes in seeds for effectively scavenging salinization stress-induced ROS, and consequently improving their salinization stress tolerance ability during seed germination (Yan et al., 2020). Additionally, melatonin priming also promotes seed germination under salinization stress by regulating carbohydrate metabolism and enhancing reserve mobilization (Zhao et al., 2015; Seneviratne et al., 2019). However, due to the differences in seed parameters, the most optimum effect of melatonin priming needs to be quantified according to the target seed. Additionally, most studies have focused only on how melatonin priming affects crops and fodder grasses, but little is known about their effect on wetland species under salinization stress.

The Ramsar-listed Momoge National Nature Reserve (MNNR), a vital stopover habitat on the migration route of East Asian-Australasian migratory birds, serves as an ecological barrier in preventing the soil salinization and desertification in the Songnen plain (Tang et al., 2021; Zhang M. Y. et al., 2022). Over the past 50 years, soil salinization due to climate change and anthropogenic activities has resulted in a loss of wetland area and severely limited the ecological service functions in the MNNR (Li X. Y. et al., 2017). As an indigenous species, *Suaeda corniculata* is widely distributed in the lake, river, and saline wetlands in MNNR. It was selected as the pioneer species for vegetation restoration in this region due to their ability to absorb soluble salt from soil and consequently increase their organic matter content (Zhao et al., 2003; Yang et al., 2017; Wang D. W. et al., 2020). Recently, the MNNR plans to not only implement the seed-based wetland restoration project, but also enhance the salinization stress tolerance ability of *S. corniculata* to improve their impact on wetland restoration. Therefore, studying the effect of melatonin priming on *S. corniculata* seed germination, antioxidant defense, and reserve mobilization is of great importance in achieving wetland restoration.

Due to the primary aim of restore degraded salinization wetlands, current studies have deepened our comprehension of sexual propagation-based restoration strategies. However, little is known about the positive effects of improve stress tolerance of germinating seeds by priming treatment (Wang X. Y. et al., 2020; Zhao et al., 2021b). In this study, laboratory experiment was carried out to investigate the effects of melatonin priming on *S. corniculata* seed germination, antioxidant enzyme activity, and reserve mobilization. The aims of this study are: (1) to examine the response of *S. corniculata* seed germination to melatonin priming; (2) to identify the effect of melatonin priming on *S. corniculata* seed's antioxidant enzyme activity and reserve mobilization; and (3) to reveal the tolerance regulatory pathways induced by melatonin priming during *S. corniculata* seed germination under salinization stress conditions. We hypothesized that the accelerating effect of exogenous melatonin happens in a concentration-dependent manner during *S. corniculata* seed germination under salinization stress. This study will assist facilitate the understanding of the

protective effects of seed priming under abiotic stress conditions, and also provide technique guidelines for salinized wetland restoration in the MNNR.

MATERIALS AND METHODS

Seed Materials

Matures seeds were collected in late September 2020 from the wild *S. corniculata* populations in the Ertou wetlands in the MNNR ($45°53'$ – $45°55'$ N, $123°36'$ – $123°41'$ E). The collected seeds were air-dried indoors under dark conditions and then stored in cloth bags at $4°C$ until the start of the experiment in January 2022. Healthy *S. corniculata* seeds were surface-sterilized using 75% ethanol for 10 min and then rinsed 9 times with distilled water, and the water was wiped off the seeds surface with sterile filter paper in the super clean bench before being used in the experiments.

Experimental Design

The experiment was conducted in the Key Laboratory of Wetland Ecology and Environment of the Northeast Institute of Geography and Agroecology, Chinese Academy of Sciences. We selected six priming treatments: MT10 (10 µM melatonin), MT20 (20 µM melatonin), MT50 (50 µM melatonin), MT100 (100 µM melatonin), MT200 (200 µM melatonin), and CK (distilled water). Seeds were incubated in each priming solution for 12 h. For each treatment, 25 seeds were evenly placed in Petri dishes (diameter 9 cm) containing double Whatman No. 1 filter paper soaked with 2 ml of 30 mM NaHCO₃ solution (the salt solution concentration data comes from the MNNR), and sealed with Parafilm® to prevent moisture evaporation. The seeds were then germinated in an artificial climate box (RDN-300B-4, China) under an alternating cycle of 12/12 h light and dark at an average temperature of $22.5/8.6°C$ (the climate data represents field conditions in May in the MNNR). For each germination treatment, five independent replicates were used (three petri dishes were used to test the germination rate of *S. corniculata* seeds, while two petri dishes were used to cultivate testable seed materials). Germinated seeds were recorded daily, and germination was considered having occurred when the radicle $\geq$ 2 mm. The entire experiment lasted for 7 days (from 6th to 12th January, 2022) and partial seeds were harvested on days 1, 3, 5, and 7, and immediately frozen in liquid nitrogen and stored at $-80°C$ until further analysis of antioxidant enzymes and reserve substances.

Determination of Germination

In order to describe seed germination changes, the germination rate (GR), germination speed (GS), and germination index (GI), were measured according to following Biju et al. (2017):

$$\text{GR} = \frac{n}{N} \times 100\% \tag{1}$$

$$\text{GS} = \left(N_1 + \frac{N_2 - N_1}{2} + \frac{N_3 - N_2}{3} + ... + \frac{N_t - N_{t-1}}{t}\right) \times 100 \tag{2}$$

$$\text{GI} = \sum \frac{G_t}{D_t} \tag{3}$$

where n is the number of germinated seeds, N is the total number of the tested seeds, Nt is the percentage of germinated seeds at the tth days, G_t is the number of germinated seeds in t days, and Dt is the corresponding germination days.

Determination of Antioxidant Enzyme Activity and Reserve Content

The activities of superoxide (SOD), peroxidase (POD), and the malondialdehyde (MDA) content were determined via WST-8, visible-spectrophotometry and TBA-spectrophotometry by using detection kits (M0102B, M0105B, M0106B, Michy Biomedical Technology Co., Ltd., Suzhou, China), respectively. The contents of starch (ST), soluble sugar (SS), and soluble protein (SP) were determined by detection kits (M1101B, M1503B, M1806B, Michy Biomedical Technology Co., Ltd., Suzhou, China) which are based on anthrone-sulfuric acid colorimetry, anthrone colorimetry, and Lowry's method, respectively. The fat content was determined by the supercritical fluid-Soxhlet extractor using petroleum ether as the extraction buffer (Soriano et al., 2011). All the samples were conducted in three independent biological replicates.

Statistical Analysis

Statistical analysis was conducted using SPSS 22.0 (SPSS, Chicago, IL, United States) and Origin Pro 2022 (OriginLab, Northampton, MA, United States). The data normality and homogeneity were checked before further analyses; seed GR, SOD, and ST were log-transformed and square root transformed to meet the assumptions of homoscedasticity. Effects of melatonin priming on *S. corniculata* seeds under salinization stress were evaluated using one-way analysis of variance (ANOVA). Duncan's multiple comparison test was used to determine the significant differences at the 0.05 significance level. Pearson's correlation analysis was performed to identify the correlational relationship between antioxidant enzymes, reserve contents, as well as seed germination. Structural equation model (SEM) analysis was performed to evaluate the direct and indirect regulatory pathways of melatonin priming on *S. corniculata* seed germination in SPSSAU[1].

RESULTS

Seed Germination Characteristics

Among the different priming treatments, we observed significant differences in the *S. corniculata* seed GR ($F = 20.64$, $p < 0.05$), ranging from 51.33 to 87.33%, with the maximum and minimum being recorded in the M50 and M200 treatments, respectively (**Figure 1A**). Priming treatment significantly affected the GS ($F = 13.20$, $p < 0.05$), which peaked in M50 (7.47) and was 1.80 times greater than in the M200 treatment (**Figure 1B**). Additionally, the GI was significantly affected by the priming treatments ($F = 19.75$, $p < 0.05$). The GI values
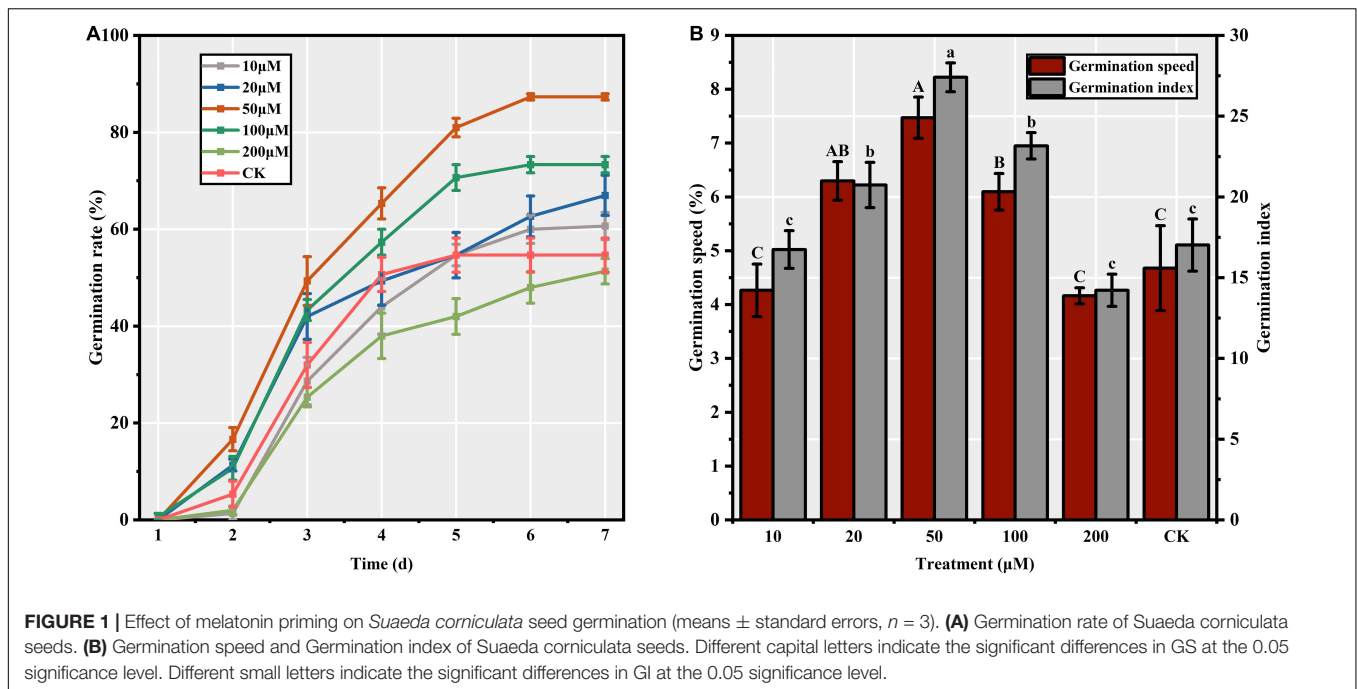
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[1]www.spssau.com

FIGURE 1 | Effect of melatonin priming on *Suaeda corniculata* seed germination (means ± standard errors, *n* = 3). **(A)** Germination rate of Suaeda corniculata seeds. **(B)** Germination speed and Germination index of Suaeda corniculata seeds. Different capital letters indicate the significant differences in GS at the 0.05 significance level. Different small letters indicate the significant differences in GI at the 0.05 significance level.

recorded in the M50 treatment (27.41 ± 0.89) were 61.05 and 92.28% higher than those in CK and M200 treatment, respectively (**Figure 1B**).

Antioxidant Enzymes Activity and Malondialdehyde Content

We identified significant differences in the activities of antioxidant enzymes of *S. corniculata* seeds at each stage under different melatonin priming treatments (**Table 1** and **Figure 2**). The antioxidant enzymes activities showed a gradually increasing trend during germination stage, with both SOD and POD activities peaking in the M50 treatment on the 7th day, and which were 2.59 and 3.67 times greater than the M200 treatment during the same period (**Figures 2D,H**). The degree of membrane lipid peroxidation in *S. corniculata* seeds was significantly affected by the melatonin priming treatments (**Figure 2**). The MDA content gradually decreased with germination time, with its lowest value appearing in the M50 treatment on the 7th day, and it was 27.33% lower than that on the 1st day (**Figures 2I,L**).

Reserve Mobilization

Melatonin priming significantly affected the reserve mobilization process in *S. corniculata* seeds (**Table 1**). The trend of changes in the starch and fat contents was basically uniform across each stage, i.e., they decreased significantly with the increase in germination days, where the seed starch and fat contents under M50 treatment decreasing up to 40.36 and 8.87%, respectively (**Figures 3A,D**). The soluble sugar content continuously increased with the germination stage, with the increase ranging from 13.90 to 56.11% under each melatonin treatment (**Figure 3B**). Additionally, the soluble protein content initially increased before decreasing during the process of

S. corniculata seed germination (**Figure 3C**). By the 7th day, the soluble protein content of the M200 treatment was the highest, thus indicating that its mobilization level was significantly lower than in the other treatments.

The Coupling Relationships of Seed Germination, Antioxidant Enzyme Activity, and Reserve Mobilization Under Melatonin Priming Treatments

We observed significant positive relationships between SOD, POD, and soluble sugar, while there were significant negative relationships with MDA, starch, soluble protein, and fat (**Figure 4**). Additionally, the GR was significantly correlated with antioxidant enzymes' activity and reserve mobilization. The SEM analysis showed that the differences in melatonin concentration priming were directly related to the antioxidant enzymes' activity, reserve mobilization, and seed germination (**Figure 5**). Meanwhile, melatonin priming also indirectly affected *S. corniculata* seed germination by affecting their antioxidant enzymes' activity and reserve mobilization. Additionally, the degree of reserve mobilization directly affected the level of antioxidant enzymes' activity.

DISCUSSION

Since soil salinization had caused losses of ecological functions and also in wetland area in the MNNR, the process of effectively restoring degraded wetlands has attracted a lot of attention from the wetland manager and policymakers (Zhao et al., 2021a). Priming treatment, a key technique to alleviate the effects of abiotic stress on seed germination, had been attempted

TABLE 1 | Result (*F* and *p*-values) of one-way ANOVAs about the priming with melatonin effects on *Suaeda corniculata* seed antioxidant enzymes activity and reserve traits.

Traits	1st day		3rd day		5th day		7th day	
	F	p	F	p	F	p	F	p
SOD	8.794	0.000***	32.911	0.000***	23.023	0.000***	9.898	0.000***
POD	9.319	0.000***	5.148	0.002**	21.700	0.000***	20.397	0.000***
MDA	3.592	0.013*	5.479	0.001**	2.802	0.046*	10.054	0.000***
ST	0.969	0.454	0.440	0.817	0.292	0.913	2.187	0.040*
SS	3.802	0.010*	0.525	0.755	0.618	0.687	3.316	0.039*
SP	2.824	0.035*	0.406	0.840	0.660	0.657	4.893	0.003**
FA	1.409	0.253	1.090	0.388	1.774	0.152	8.189	0.000***

SOD, superoxide dismutase; POD, peroxidase; MDA, malondialdehyde; ST, starch; SS, soluble sugar; SP, soluble protein; FA, fat; *p < 0.05; **p < 0.01; ***p < 0.001.
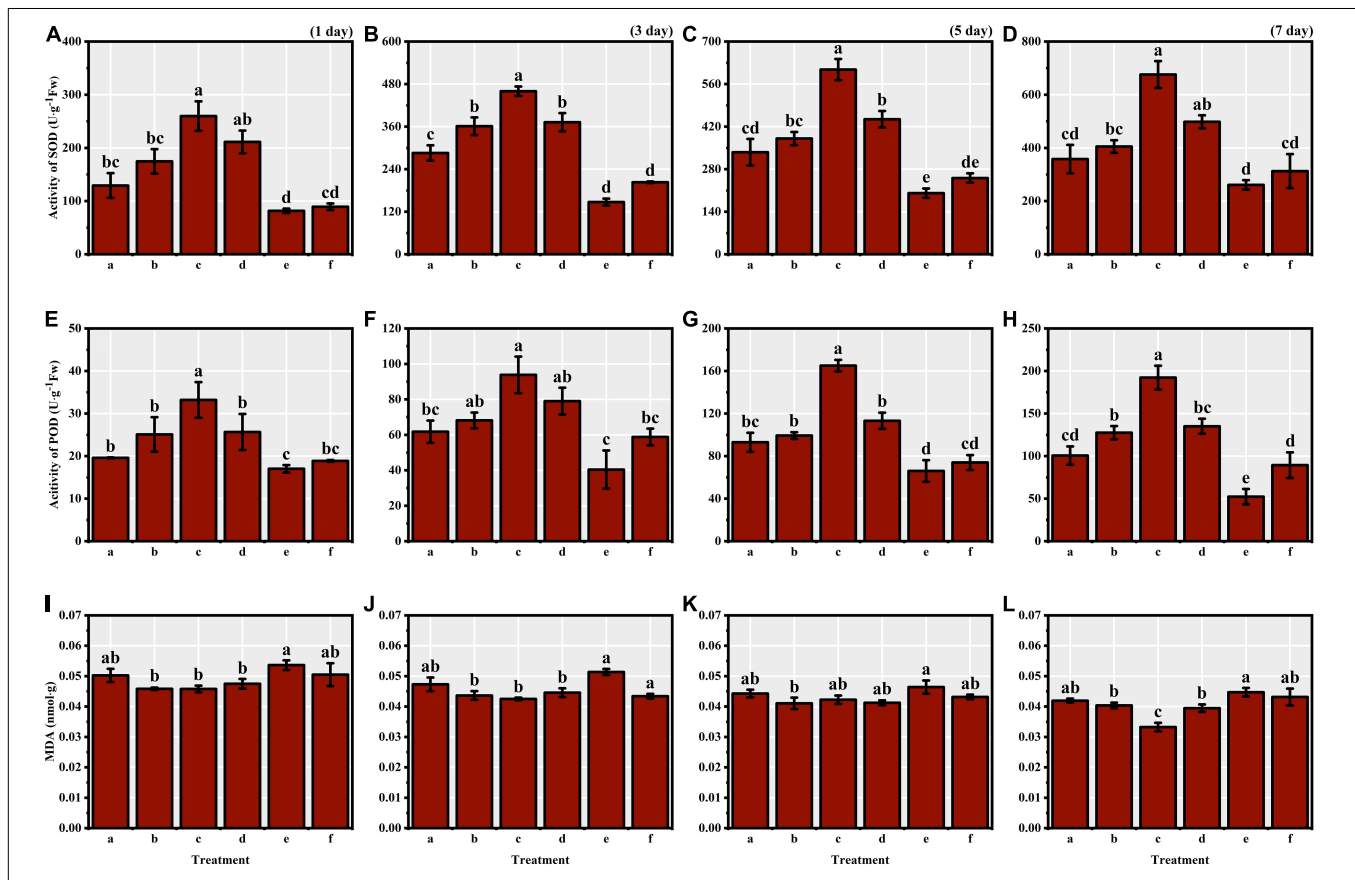

FIGURE 2 | The antioxidant enzymes' activity and MDA content of *Suaeda corniculata* seeds under melatonin priming during the entire germination period (means ± standard errors, *n* = 3). Different letters indicate the significant differences at the 0.05 significance level. SOD, superoxide dismutase; POD, peroxidase; MDA, malondialdehyde; (a) M10; (b) M20; (c) M50; (d) M100; (e) M200; (f) CK. **(A)** SOD of 1st day; **(B)** SOD of 3th day; **(C)** SOD of 5th day; **(D)** SOD of 7th day; **(E)** POD of 1st day; **(F)** POD of 3th day; **(G)** POD of 5th day; **(H)** POD of 7th day; **(I)** MDA of 1st day; **(J)** MDA of 3th day; **(K)** MDA of 5th day; **(L)** MDA of 7th day.

recently in wetland restoration engineering (Zhang M. Y. et al., 2021). It boosts the antioxidant and DNA repair systems, and also modulates the reserve mobilization for improving seed germination (Yan et al., 2020; Forti et al., 2021). Our results support the notion of effectively applying the priming treatment to wetland plant seed germination, and thus confirm the previously proposed hypothesis that melatonin priming had a concentration-dependent effect on *S. corniculata* seed germination during salinization stress. Priming with M50 had

significantly positive effects on the seed germination, antioxidant defense, and reserve mobilization of *S. corniculata*.

Effect of Melatonin Priming on the Seed Germination of *Suaeda corniculata*

Seed propagation is preferred for maintaining plant genetic variation, and it will also play an important role in the process of near-naturalized wetland restoration (Kim, 2019). The seed
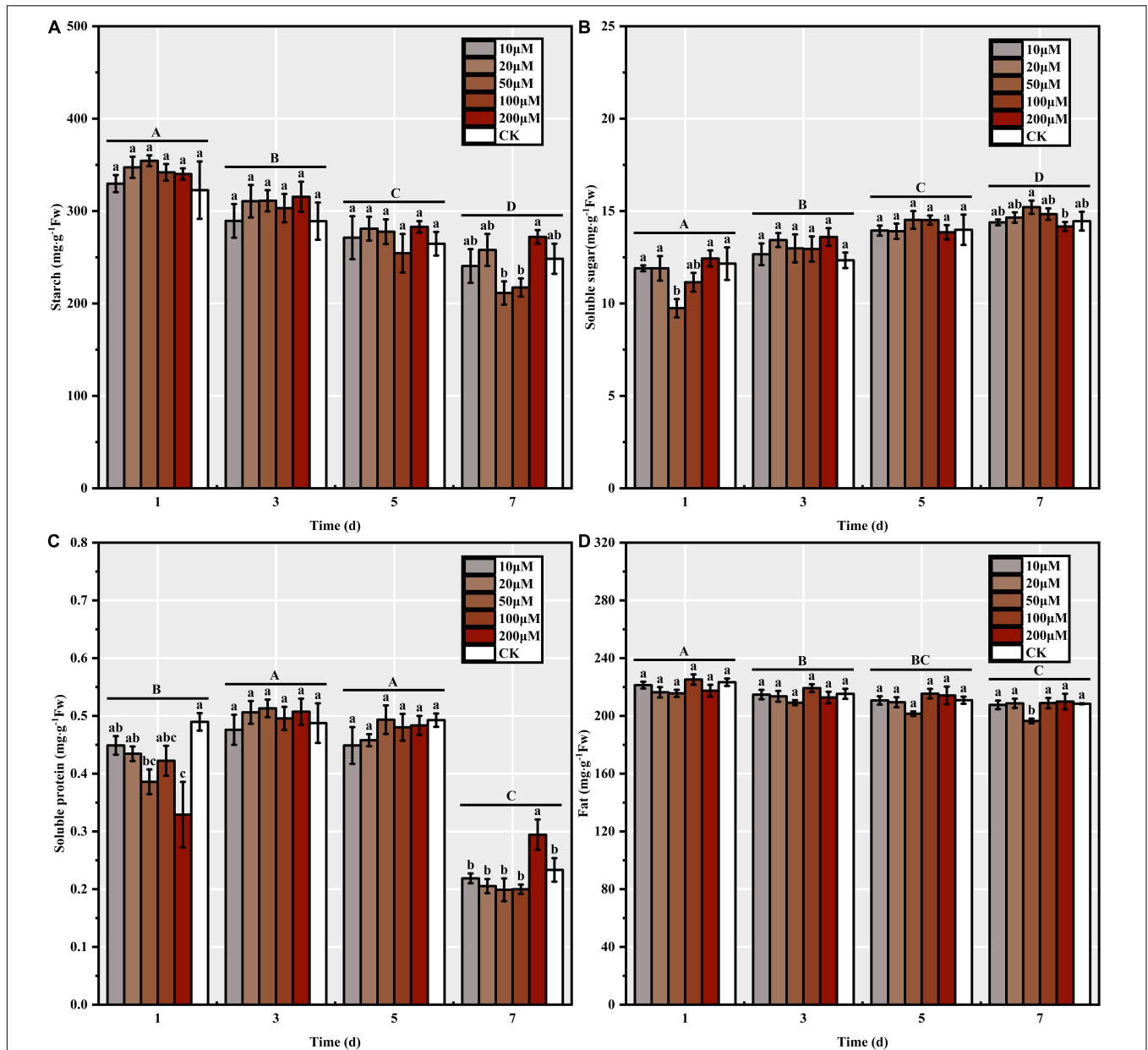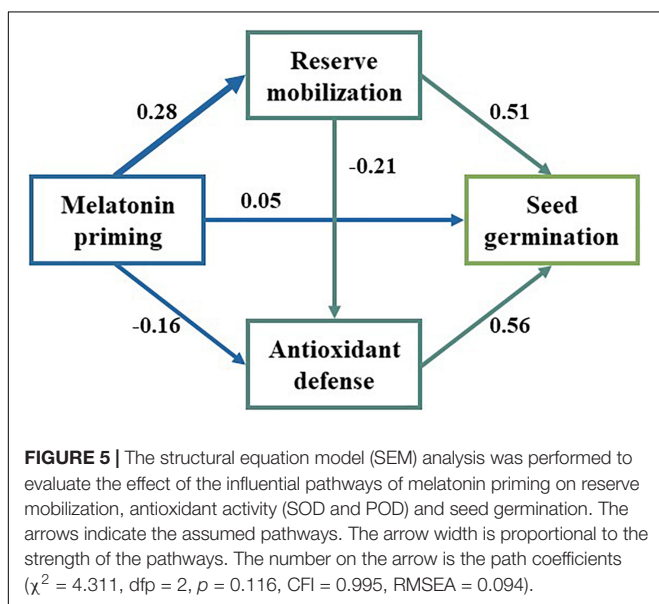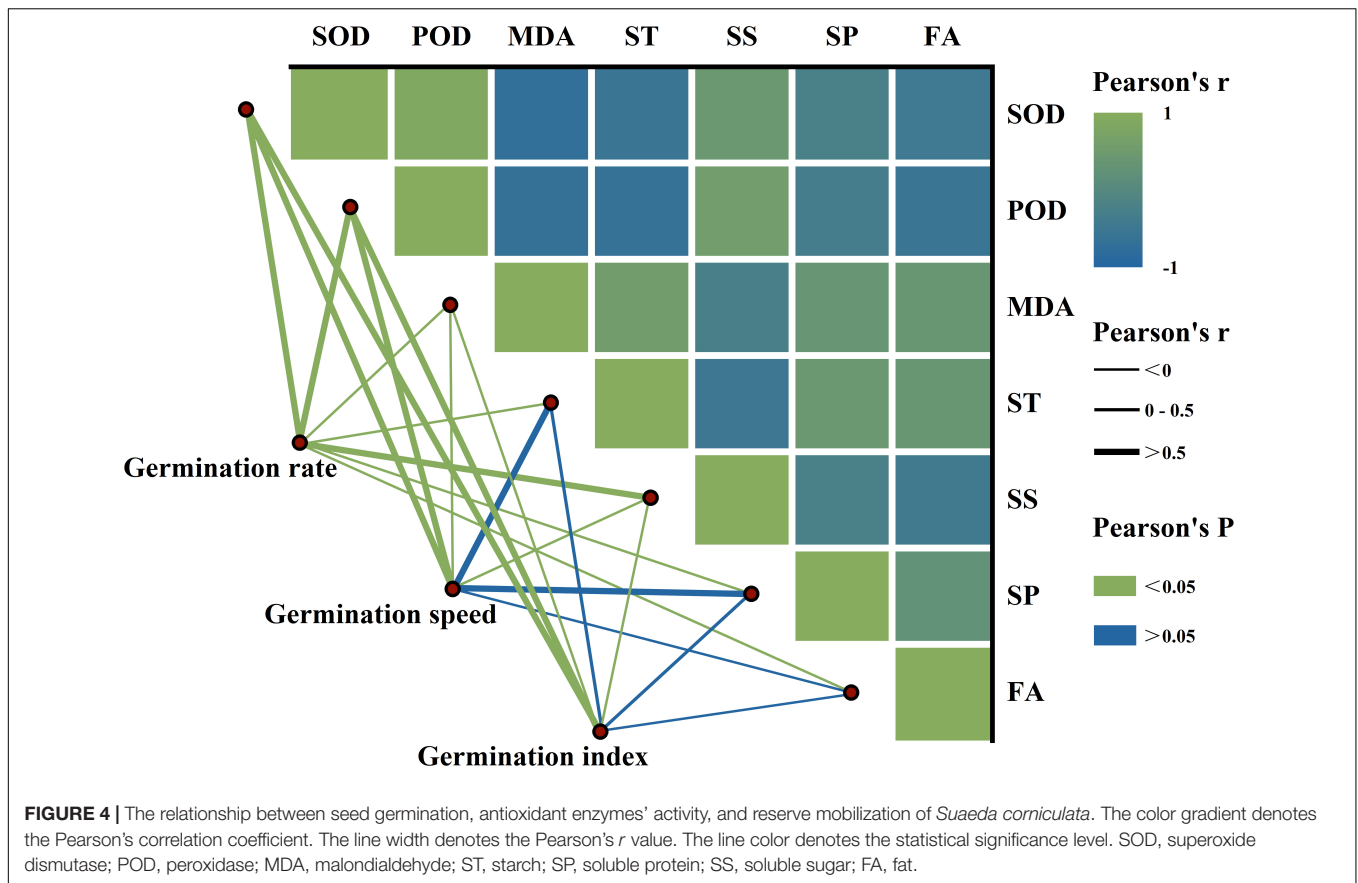
FIGURE 3 | The reserve mobilization of *Suaeda corniculata* seeds under melatonin priming during the entire germination period (means ± standard errors, *n* = 3). **(A)** Starch, **(B)** Soluble sugar, **(C)** Soluble protein, and **(D)** Fat of *Suaeda corniculata* seeds. Different capital letters indicate significant differences in the germination stages at the 0.05 significance level; different small letters indicate significant differences in the treatments at the 0.05 significance level.

germination process is usually affected by salinization stress, which leads to the decrease in germination quality and even results in an incomplete plant life cycle (Jia et al., 2018). Multiple studies have shown that melatonin is a major regulator not only involved in seed germination, but also in diverse physiological and biochemical processes like stress resistance-related gene expression, boosting the antioxidant defense system, and reserve mobilization (Cao et al., 2019; Chen et al., 2021; Zhang T. G. et al., 2022). Our results showed that melatonin priming positively affected seed germination of *S. corniculata* in a

concentration-dependent manner, while significantly increasing the GR, GS, and GI of *S. corniculata* seed under the M50 treatment. This finding was consistent with Qin and Zeevaart (2002) and Ogawa et al. (2003), where they found that exogenous melatonin priming upregulates the expression of gibberellins (GA) biosynthesis genes (GA20ox and GA30ox), while downregulating the key abscisic acid (ABA) biosynthesis genes (LbNCED1 and LbNCED3), both of which affect seed germination. Additionally, the appropriate melatonin priming concentration activated the SOS pathway to improve the seed's

FIGURE 4 | The relationship between seed germination, antioxidant enzymes' activity, and reserve mobilization of *Suaeda corniculata*. The color gradient denotes the Pearson's correlation coefficient. The line width denotes the Pearson's *r* value. The line color denotes the statistical significance level. SOD, superoxide dismutase; POD, peroxidase; MDA, malondialdehyde; ST, starch; SP, soluble protein; SS, soluble sugar; FA, fat.

FIGURE 5 | The structural equation model (SEM) analysis was performed to evaluate the effect of the influential pathways of melatonin priming on reserve mobilization, antioxidant activity (SOD and POD) and seed germination. The arrows indicate the assumed pathways. The arrow width is proportional to the strength of the pathways. The number on the arrow is the path coefficients ($\chi^2 = 4.311$, dfp = 2, $p = 0.116$, CFI = 0.995, RMSEA = 0.094).

hormone signal pathways in response to salinization stress (Zhang Y. X. et al., 2021). However, the mitigative effect of melatonin priming treatment on salinization stress could be counterproductive at high concentration. Our results suggested that the M200 treatment inhibited the *S. corniculata* seed germination as compared with the CK treatment. This may be because high melatonin concentration promoted the synthesis of endogenous ABA, which regulated seed germination via ABI3, ABI4, and ABI5 and promotes dormancy via the response factors like SCHNARCHZAPFEN (SNZ) and ABA overly sensitive 5 (ABO5) (Liu et al., 2010; Zou et al., 2013). This explanation has been supported by the fact that 1,000 μM melatonin treatment significantly inhibited the *Arabidopsis* seed germination as compared with the untreated control in Lv et al. (2021).

Effect of Melatonin Priming on Antioxidant Defense of *Suaeda corniculata* Seeds

The excessive accumulation of reactive oxygen species (ROS) not only has irreversible negative effects on seed germination, but also affects the future seedling growth (Goud and Kachole, 2011). Antioxidant system is one of the major physiological defense systems protecting against salinization-induced oxidative stress by improving the antioxidant enzyme activity and antioxidant contents (Ibrahim, 2016). In this sense, studying the antioxidant defense is crucial for a holistic understanding

salinity tolerance at the germination stage by upregulating the expression of chloride channel proteins (CLC1 and CLC2) (Li X. J. et al., 2017). Meanwhile, priming with the appropriate melatonin concentration could up-regulate the ethylene, kinetin, and brassinolide biosynthetic gene expression and the plant

of priming treatments. Previous studies have demonstrated that melatonin get through the free radical scavenging cascade, extends the free radical scavenging capacity of antioxidants to its multi-stage metabolites. Especially N1-acetyl-N2-formyl-5-methoxykynuramine, a primary metabolite derived from melatonin, also has strong antioxidant capacity, thus effectively protecting organisms from oxidative stress (Balabusta et al., 2016). Simultaneously, melatonin helps scavenge free radicals through its receptors by modulating the antioxidant enzymes in the cells and tissues in a dose-dependent manner (Li et al., 2018; Cao et al., 2019). Our results showed that the SOD and POD activities of *S. corniculata* seeds continued increasing with the germination stage, which was significantly higher than others in the M50 treatment. Exogenous melatonin priming may have indirectly upregulated the expression of antioxidant enzyme and related genes, which then improves their efficiency as antioxidants (Arora and Bhatla, 2017; Wang et al., 2017). However, other studies have demonstrated that exogenous melatonin priming did not change the antioxidant enzymes' activity of *Lolium perenne* (Zhang et al., 2016; Lu et al., 2022), and we suspect that melatonin possibly uses different physiological mechanisms to strengthen the antioxidant defense. MDA is the indicator of oxidative-stress induced membrane damage under salinization stress (Ibrahim, 2016). Our study results showed the MDA content in *S. corniculata* seeds continuously decreased during the experiment, and it decreased up to 27.33% in the M50 treatment. This result was in agreement with previous findings indicating that a negative correlation exists between the MDA content and the activity of antioxidant enzymes (Esfandiari et al., 2008; Younesi and Moradi, 2015). The possible explanation is that melatonin treatment increases the autophagy induction capacity and thereby reduces the accumulation of oxidized proteins, while also simultaneously controlling the ROS accumulation by upregulating the antioxidant enzymes activity and their transcript levels to mitigate membrane lipid peroxidation under salinization stress (Wang et al., 2015; Sun et al., 2020; Zhang T. G. et al., 2022). Additionally, it improves the activity of non-enzymatic antioxidants by regulating the activity of enzymes in the ASA-GSH cycle, thereby further improving the free radical scavenging capacity (Ni et al., 2018). Simultaneously, by up-regulating the genes related to glutathione metabolic pathway and activating the downstream signal transduction pathway, also may help explain how melatonin priming improves the antioxidant defense of seeds under salinization stress (Zhang Y. X. et al., 2021).

Effect of Melatonin Priming on Reserve Mobilization of *Suaeda corniculata* Seeds

It is well known that the seed reserve is an important factor which affects germination characteristics, and reserve mobilization plays an important role in the energy supply and maintains osmotic balance (Zhao et al., 2018; Lei et al., 2021). Recent experimental data suggested that the priming treatment regulates the carbohydrate, protein, and fat metabolism, thereby resulting

in increased seed germination (Li et al., 2019; Chen et al., 2021). However, the effect of melatonin priming treatment on reserve mobilization under abiotic stress still needs to be studied according to the target species. Our results showed that there is a strong negative correlation between starch and soluble sugar content variation. A plausible explanation for this phenomenon could be that melatonin priming upregulated the α-amylase gene expression, to ensure that starch is broken down into smaller and simpler molecules which will be used as an energy source by the organism for seed germination (Cao et al., 2019; Yan et al., 2020). Previous studies also found that the priming treatment induced a phased effect on the change of seed soluble protein content under abiotic stress (Adawy et al., 2003). Priming treatment induced seeds to adopt the defensive strategy of accumulating soluble protein for improving the cell's water holding capacity and protect its membrane (Li et al., 2010). During the later stage of germination, hydrolysis of storage proteins provides the energy for both the hypocotyl growth and new protein synthesis (Wahid and Bounoua, 2013). Meanwhile, the fat metabolism also pushed the seed's developmental switch from dormancy to germination (Footitt et al., 2002), which was similar to our data.

Pathways of Melatonin Priming in *Suaeda corniculata* Seed Germination

The action pathway of priming treatment is the basis for revealing the driving mechanisms. The SEM analysis showed that there are both direct and indirect effect pathways of melatonin priming on the *S. corniculata* seed germination under salinization stress conditions (**Figure 5**). On the one hand, melatonin priming promoted seed germination by regulating the expression of stress tolerance, hormone synthesis, and metabolism-related genes (Zhang Y. et al., 2021). On the other hand, priming treatment also upregulated the antioxidant enzyme activities and accelerated the level of reserve mobilization, ultimately improving the seed germination (Arora and Bhatla, 2017; Cao et al., 2019; Lu et al., 2022). This study is the first to reveal the effect pathways of melatonin priming on the *S. corniculata* seed germination under salinization stress, and also proved the feasibility of melatonin priming in improving the germination of *S. corniculata* seeds. It is also worth noting that the positive effects of the melatonin priming we found were concentration-dependent, which reconfirms our hypothesis.

CONCLUSION

In summary, we comprehensively assessed the effect of melatonin priming on the *S. corniculata* seed germination under salinization stress. Our results showed that the stress-alleviating effect of melatonin priming is concentration-dependent. The melatonin priming concentration of 50 μM significantly improved the germination characteristics of *S. corniculata* seeds, increased the activity of antioxidant enzymes to alleviate the salinity stress-induced membrane lipid peroxidation, and promoted the reserve mobilization during germination. However, the treatment of 200 μM melatonin inhibited the above mentioned physiological activities. Melatonin priming directly influences

the seed germination of *S. corniculata*, while also acting *via* indirect pathways to regulate the antioxidant defense and reserve mobilization. Our study is the first to explore the effects of exogenous melatonin on *S. corniculata* seed germination, antioxidant defense, and reserve mobilization under salinization stress, thereby providing invaluable information and technical support for the restoration of salinized wetlands. However, further studies are required to understand the synthetic effects of salinization stress intensity on the *S. corniculata* seed germination post-priming treatments, before a general conclusion can be drawn.

DATA AVAILABILITY STATEMENT

The original contributions presented in this study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author.

AUTHOR CONTRIBUTIONS

MZ, ST, and XW designed the study. MZ, SL, and YW collected the data. DZ, QQ, and YA analyzed the data. ST and XL lead the writing with all co-authors. All authors gave final approval for publication.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2022.941032/full#supplementary-material

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